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The Hygrophoraceae of Tasmania

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Abstract

Twenty-six taxa of Tasmanian Hygrophoraceae are listed or described. New taxa are: *Hygrocybe franklinensis* A.M.Young & A.K.Mills, *Hygrocybe roseoflavida* A.M.Young & A.K.Mills and *Hygrophorus involutus* var. *albus* A.M.Young & A.K.Mills. Full descriptions and diagrams are provided where necessary for all new taxa. Previously described taxa found in Tasmania are listed together with details of any new information and all known herbarium collections for the Tasmanian species. Keys to the species are included.

Introduction

Little has been published on the Hygrophoraceae of Tasmania other than as minor inclusions in publications that relate to other mycological or botanical investigations. Massee (1899) described the new species *Hygrophorus rodwayi* Massee from a location near Hobart but further information on Tasmanian Hygrophoraceae was not published for a little over 90 years. Young and Wood (1997) commented on the probable richness of the Tasmanian flora within family Hygrophoraceae as suggested by the work of Monks (1989) while Young (2000a) indicated that Tasmanian collections had recently been made of both *Hygrocybe rodwayi* (Massee) A.M.Young and *H. lewelliniae* (Kalchbr.) Brittleb. *ex* A.M.Young.

A recent paper dealing solely with the Tasmanian Hygrophoraceae is that of Monks and Mills (1991) who provided a more concise description of *Camarophyllus rodwayi* (Massee) Monks & A.K.Mills. This was followed by the description of the new species *Hygrocybe erythrocrenata* Monks & A.K.Mills and the demonstration of coloured spore prints for both this new species and *Hygrocybe lilaceolamellata* (G.Stev.) E.Horak (Mills & Monks 1993). A small but very beautiful collection of coloured photographs of Tasmanian Hygrophoraceae is contained in the booklet on Tasmanian rainforest fungi (Fuhrer & Robinson 1992). Unfortunately, little other information is provided, however many of the 16 taxa depicted are easily recognisable, excellent definitive illustrations for these species are provided, and are cited as such within this paper.

There is no longer any doubt as to the large number of species of Hygrophoraceae in Tasmania; however this paper should be considered only as a preliminary survey of the Tasmanian taxa. Although three new taxa are described, the paper's principal aim is to provide a foundation for future studies on the Tasmanian Hygrophoraceae by providing a census of previously described taxa that occur in Tasmania together with any pertinent data. Collections made by both authors during the seasons of 1998 and 1999 indicate that a considerable number of taxa remain to be formally described.

The Tasmanian species of Hygrophoraceae differ considerably in known habitat when compared to the mainland flora. Mainland taxa are found in a wide variety of habitats which include grassland, heath, dry sclerophyll woodland, dry and wet sclerophyll forest, various forms of rainforest and beech forest (*Nothofagus* spp.). This is not the case for Tasmanian taxa which are almost exclusively found in cool temperate rainforests that are usually dominated by *Nothofagus cunninghamii* (southern beech) and *Artherosperma moschatum* (sassafras) with an under-story of tree ferns (*Cyathea* and *Dicksonia* spp.).

The floors of these forests contain not only abundant, moist litter, but also immense carpets of moss, and the combination of these two substrates (together with the conditions of temperature and humidity maintained under the forest canopy) seems to be particularly suitable for the fruiting of species of Hygrophoraceae. Assuming the normal seasonal rainfall, some species commence to produce fruiting bodies in early April but a maximum of both species fruiting and basidiomes produced by each species occurs during May and early June. Some species continue to produce considerable numbers of fruiting bodies in July and a few species may still produce scattered basidiomes as late as mid-August.

Two instances where taxa occurred outside the cool temperate rainforest were noted during 1999 when *Hygrocybe cantharellus* (Schwein.) Murrill was collected from amongst moss in heath and a small collection of an undescribed species of *Hygrocybe* was found on a moss bank beside a path in coastal eucalypt forest. Extensive collecting by the second author has demonstrated that when taxa of Hygrophoraceae have been collected in the Tasmanian wet sclerophyll forests (dominated by *Eucalyptus* spp.), then the collection sites are marginal zones where cool temperate rainforest tree species intermingle with the wet sclerophyll forest species. There are no known collections of Tasmanian Hygrophoraceae from that state's dry sclerophyll forests and only a single collection from pasture is here recorded.

Generally, the basidiomes of species as they occur in Tasmania, have characteristics which conform very closely to the known characters of the taxa as they occur on the Australian mainland, however they do occasionally differ. Spore sizes in some Tasmanian Hygrophoraceae are sometimes larger than those of either or both the holotype or the Australian mainland representatives of the respective species. This is true for the taxa *Hygrocybe cheelii* A.M.Young and *Hygrocybe irrigata* (Pers.: Fr.) Bon. Other microscopic differences observed include much longer basidia in basidiomes of material currently accepted as *Hygrocybe stevensoniae* T.W.May & A.E.Wood and spore shape variations in both *Hygrocybe rodwayi* and *Hygrocybe astatogala* (R.Heim) Heinem.

Biogeographically, the Tasmanian species of Hygrophoraceae also show relationships with the Hygrophoracee of New Zealand. A number of taxa occur in both geographical locations and New Zealand is the holotype location for several species. Links with South America are less well defined, however the species *Hygrocybe reesiae* A.M.Young is undoubtedly related to the South American taxon *Camarophyllus adonis* Singer. Several taxa such as *Hygrocybe cantharellus* and *H. miniata* (Fr.: Fr.) P.Kumm. are cosmopolitan, although first described from Europe, but others such as *H. astatogala* have tropical distributions.

Materials and Methods

All material collected in Tasmania during 1998–1999 was air dried and preserved for later microscopic examination. Field notes were made from the fresh material and the accompanying colour codes refer to Kornerup and Wanscher (1981). Photographic slides were made either in the field or in the laboratory. The 1998–1999 material collected by the first author forms the basis for this paper supplemented with material from the second author's herbarium. Distribution notes for each taxon are limited to Tasmania, however many of the taxa occur on the Australian mainland and in New Zealand.

The Tasmanian material (other than holotypes) cited from the 1998–1999 collections has been deposited in either the Queensland State Herbarium (BRI) or the National Herbarium of Victoria (MEL). Holotype collections and all cited material from the Mills collection have been deposited in the Tasmanian Herbarium (HO). Collection numbers from each author's personal herbarium (cited as '*hb. Young*' and '*hb. Mills*' respectively) are provided for reference purposes. Material was also studied from AD, K, MICH, PDD, UNSW, ZT (Holmgren *et al.* 1990). Material in collections with ZT numbers has been divided: part remains in ZT and part is in BRI.

All microscope work was done on an Olympus CX40 binocular microscope with drawing tube attachment. The drawing tube was calibrated to provide scale drawings using an Olympus standard 1 mm slide. Material intended for microscopic analysis was rehydrated in ammoniated congo-red and gently warmed if necessary.

Illustrations are provided for the new taxa and for those species which are either not illustrated in previous papers (Young & Wood 1997; Young 1999; Young 2000a; Young *et al.* 2000) or which require additional diagrams as a result of new information. The habit-sketch shows basidiome dimensions. The microstructures of the pileus, hymenophoral trama and stipe are not depicted because they conform to standard forms (Young & Wood 1997). For each illustrated specimen, 20 spores and 10 basidia were selected at random, drawn and measured. The derived parameter 'Q' is defined as the quotient of the length divided by the width of the relevant spore or basidium; the mean 'Q' is the quotient of the mean length and width respectively.

This paper lists several species of Hygrophoraceae originally collected and described from Europe that are stated to have no type (Boertmann *pers. com.*). This problem has already been addressed (Young 2000a) and where types for European taxa do not exist, the species concepts of Boertmann (1995) are used.

Taxonomy

Family **Hygrophoraceae** Lotsy, *Vortr. Bot. Stammesg.* 1: 706 (1907). *Typical genus*: *Hygrophorus* Fr.

Basidiome small to medium sized, stipitate. *Pileus* conical, convex, umbilicate or infundibuliform; sometimes perforate; surface dry, moist, viscid or glutinous, smooth to squamulose or fibrillose. *Lamellae* generally thick, waxy, and distant; free or adnexed to decurrent. *Stipe* central, often brittle, with similar surface moisture or structures to pileus. *Universal veil* generally absent. *Context* soft, frequently thin, waxy and translucent. *Spore print* white, cream, pale violaceous or magenta. *Spores* small to large, subglobose to ovoid, ellipsoid or cylindrical, sometimes constricted, smooth, more rarely nodulose or echinulate, hyaline or rarely with dark contents, inamyloid rarely amyloid. *Basidia* often long and narrow. *Cheilocystidia* sometimes present, pleurocystidia rare and then as pseudo-pleurocystidia. *Hymenophoral trama* regular, irregular or bilateral. *Pileipellis* a cutis, trichoderm, ixocutis or ixotrichoderm, rarely a hymeniderm or epithelium. *Clamp connections* present or absent. Development gymnocarpic, occasionally hemiangiocarpic. Terrestrial, rarely lignicolous, mycorrhizal or saprobic.

Key to the tribes of Hygrophoraceae

1. Lamellae with regular to irregular trama, never divergent .....**Tribe 1. Hygrocybeae**
1. Lamellae with divergent trama .....**Tribe 2. Hygrophoreae**

TRIBE 1. HYGROCYBEAE Kühner, *Bull. mens. Soc. linn. Lyon* 48: 621 (1979). *Typical genus*: *Hygrocybe* (Fr.) P.Kumm.
Hymenophoral trama regular to irregular; not forming ectomycorrhizae.

Key to the genera of Hygrocybeae

1. Pileipellis composed of hyphae forming a cutis, ixocutis, trichoderm or ixotrichoderm of non-inflated, hyphal elements .....**Genus 1. *Hygrocybe***
1. Pileipellis an hymeniderm but sometimes approaching an epithelium and then composed of inflated elements (one species known for Tasmania) ....**Genus 2. *Camarophyllopsis***

GENUS 1. *HYGROCYBE* (Fr.) P.Kumm., *Führ. Pilzk.*: 26 (1871); *Hygrocybe* Fr., *Syst. Mycol.* 1: 101 (1821); *Camarophyllus* Fr., *Syst. Mycol.* 1: 98 (1821); *Camarophyllus* (Fr.) P.Kumm., *Führ. Pilzk.*: 2 (1871). *Typical species*: *Agaricus conicus* Schaeff., *Fungi Bavariae* 4: 2 (1774).

Basidiome fleshy, often watery or waxy in texture, collybioid, mycenoid or omphaloid, generally small to medium sized but occasionally large; variously coloured, often bright red, orange, yellow, green and lilac or combinations of these colours. *Pileus* opaque or hygrophanous, striate or not, dry to glutinous, smooth to squamulose or fibrillose. *Lamellae* usually sub-distant to distant, free to adnate or decurrent, thick to very thick and with waxy appearance when fresh; velar structures absent. *Universal veil* absent. *Stipe* dry to glutinous, smooth to squamulose or fibrillose; spore print white, cream coloured, pale magenta or pale lilac. *Spores* hyaline, smooth or rarely spinose, non-amyloid (for known Australian taxa). *Basidia* sometimes long (50–70 µm), Q: 2.5–10.0, 2-and 4-spored forms frequent, clamp connections usually present. *Cheilocystidia* present in some species either as true or pseudo-cheilocystidia. *Pleurocystidia* very rare and then as pseudo-pleurocystidia. *Hymenophoral trama* regular, subregular to irregular, tramal elements from very long (> 1000 µm) to very short (< 30 µm); clamp connections usually present. *Pileipellis* a cutis, ixocutis, trichoderm or ixotrichoderm. Development gymnocarpic and stipitocarpic.

Habitat and Distribution: Solitary to gregarious, terrestrial, soil, humus or moss, rarely on rotten wood; found in various ecosystems from grasslands to forest and considered to be saprobic. Cosmopolitan from subarctic or subantarctic to tropics and alpine regions.

Key to the subgenera of *Hygrocybe*

1. Hymenophoral trama irregular, composed of short (20–150 µm) interwoven hyphal elements; basidiome colours often subdued (white, brown, dull lilac-grey) but may be orange, apricot or bright lilac; lamellae arcuate to decurrent; clamps present, occasionally rare in the hymenophoral trama .....**subgen. 1. *Cuphophyllus* Key 1.**
1. Hymenophoral trama regular to subregular (if subregular, then basidiome brightly coloured) and composed of parallel hyphal elements which are either 'long tubular' or chains of short elements; basidiome often very brightly coloured (red, orange, yellow, green, lilac); lamellae variously attached; clamps present, at least at the bases of the basidia 2
2(1). Hymenophoral trama very regular, composed of very long (1000–3000 µm), aseptate, parallel, tubular elements with tapered ends; lamellae free, ascending or narrowly adnate; tissues may blacken on bruising; basidia usually short (mean length 30–40 (45) µm); except for the aseptate hymenophoral trama, clamps usually present throughout the basidiome, rarely absent in some taxa with 2-spored basidia (one species known for Tasmania) .....**subgen. 2. *Hygrocybe***
2. Hymenophoral trama regular to subregular, composed of parallel chains of short, sometimes inflated hyphal elements (usually 20–400 µm); lamellae more or less free to adnate or arto decurrent; tissues never blackening on bruising; basidia sometimes long (40–60 µm); clamps either present throughout the basidiome or present only at the bases of the basidia 3
3(2). Clamps present throughout the basidiome and of medallion form or not ....**subgen. 3. *Pseudohygrocybe* Key 2.**
3. Clamps absent throughout the basidiome except at the bases of the basidia and then frequently of medallion form.....**subgen. 4 *Humidicutis* Key 3.**

Key 1: Tasmanian species of subgenus Cuphophyllus

1. Pileus off-white to cream coloured and often with biscuit brown tints at the depressed centre ..... **4. *H. rodwayi***
1. Pileus yellow, yellow-orange or some shade of lilac or pinkish lilac 2
2(1). Pileus yellow or yellow-orange ..... **1. *H. aurantiopallens***
2. Pileus a shade of lilac to pinkish lilac 3
3(2). Pileus lilac to greyish lilac, hygrophanous; margins not involute when juvenile; stipe base lilac ..... **3. *H. reesiae***
3. Pileus bright pinkish lilac, not hygrophanous; pileus margins involute when juvenile; stipe base yellow ..... **2. *H. cheelii***

Key 2: Species of subgenus Pseudohygrocybe

1. Pileus glutinous to viscid and pileipellis always an ixotrichoderm 2
1. Pileus dry and pileipellis a cutis or trichoderm, or viscid and pileipellis an ixocutis 7
2(1). Pileus white or yellow 3
2. Pileus green, a shade of brown or grey 4
3(2). Pileus and stipe white; lamellae adnexed to narrowly adnate ..... **15. *H. leucogloea***
3. Pileus and stipe yellow; lamellae decurrent ..... **8. *H. chromolimonea***
4(2). Lamellae margins fertile and without a gluten thread; hyphal cheilocystidia absent 5
4. Lamellae margins sterile and with a gluten thread; hyphal cheilocystidia present 6
5(4). Pileus green ..... **20. *H. stevensoniae***
5. Pileus grey to grey-brown ..... **13. *H. irrigata***
6(4). Pileus green, lamellae green; spores 8.5–10.5 × 5–8 µm; dried material dull green ..... **18. *H. pseudograminicolor***
6. Pileus green to brown, lamellae white or white with green or brownish tints; spores 5.5–7.5 × 3.5–5 µm; dried material brick-pink ..... **12. *H. graminicolor***
7(1). Spores dimorphic; macrospores 11–18 µm long ..... **10. *H. firma***
7. Spores monomorphic; spores < 11 µm long 8
8(7). Pileipellis a trichoderm (at least at the centre) 9
8. Pileipellis a cutis or ixocutis 10
9(8). Lamellae deeply decurrent, cream coloured to yellowish ..... **7. *H. cantharellus***
9. Lamellae broadly adnate with at most a decurrent tooth, yellow with pink flush ..... **17. *H. miniata***
10(8). Pileus viscid; pileipellis an ixocutis 11
10. Pileus dry; pileipellis a cutis 13
11(10). Pileus conical; lamellae narrowly adnate to ascending-adnate ..... **11. *H. franklinensis***
11. Pileus convex; lamellae arcuate to decurrent 12
12(11). Lamellae pallid pink; stipe base yellow ..... **19. *H. roseoflavida***
12. Lamellae cream-coloured; stipe base orange-red or red ..... **6a. *H. anomala*** var. ***anomala***
13(10). Stipe yellow ..... **14. *H. julietae***
13. Stipe red, orange or brown 14
14(13). Pileus and stipe brown, stipe base usually mauve tinted **16. *H. lilaceolamellata***
14. Pileus red to red-brown or orange-brown, stipe red to reddish orange, stipe base concolorous 15
15(14). Lamellae delicately tinted mauve or lilac; spinose spores always present ..... **6a. *H. anomala*** var. ***ianthinomarginata***
15. Lamellae white, pinkish white, reddish grey or lilac red; spinose spores absent ..... **9. *H. erythrocrenata***

Key 3: Species of subgenus Humidicutis

1. Basidiomes wholly white .....**22. *H. mavis***
1. Basidiomes wholly pale lilac to violet .....**21. *H. lewelliniae***

Species Information and Descriptions

Subgenus 1 *Cuphophyllus* Donk, *Beih. Nova. Hedwigia* 5: 45 (1962). *Typical species*: *Agaricus pratensis* Pers.: Fr. [= *Camarophyllus pratensis* (Pers.: Fr.) P.Kumm.]

Basidiome dull coloured or rarely with bright colours in apricots, pinks or lilac to mauve; *lamellae* mostly decurrent; *hymenophoral trama* irregular; cystidia mostly absent; clamp connections frequent throughout the basidiome.

1. *Hygrocybe aurantiopallens* (E.Horak) A.M.Young *in* Young & Wood, *Austral. Syst. Bot.* 10: 921 (1997). *Camarophyllus aurantiopallens* E.Horak, *Beih. Nova Hedwigia* 43: 122 (1973). *Type*: New Zealand, Lake Rotoiti, 29.iv.1968, *E.Horak s.n.* (holotype PDD *27088*).

Misappl.: *Hygrophorus aurantius* Murrill *sensu* G.Stev., *Kew Bull.* 16: 382 (1963).
Illustrations: Fuhrer & Robinson (1992), p. 38; Young & Wood (1997), p. 922.

Habitat and distribution: gregarious on soil amongst litter in cool temperate rainforest, often at the bases of tree ferns. The species is widespread in southern, central and north-western Tasmania.

Material: Mt Field National Park, 30.iv.1998, *A.M.Young* (*hb. Young 1974*; BRI); Julius River, 6.v.1998, *A.M.Young* (*hb. Young 1985*; BRI); Milkshake Reserve, 6.v.1998, *A.M.Young* (*hb. Young 1993*; BRI); Franklin R., 7.v.1998, *A.M.Young* (*hb. Young 2001*; BRI); Tahune, 27.iv.1998, *A.M.Young* (*hb. Young 1961*; MEL *2087780*); Tahune, 11.v.1998, *A.M.Young* (*hb. Young 2012*; MEL *2087777*); Growlingswallet nr Mt Field, 19.v.1999, *A.M.Young* (*hb. Young 2227*; MEL *2087772*); Franklin R., 21.v.1999, *A.M.Young* (*hb. Young 2241*; MEL *2087769*); Cradle Mt, 3.vi.1992, *T.W.May s.n.* (MEL *259607*); Arve Loop Rd nr Geeveston, iv.1998, *A.K.Mills* (*hb. Mills 1510*; HO *508592*).

Remarks: Basidiomes of *Hygrocybe aurantiopallens* can vary in their colouration from brilliant orange to orange-yellow or apricot-yellow. Young (1999) noted the probable misidentification of Fuhrer and Robinson (1992) of a specimen considered to be *Camarophyllus apricosa* (E.Horak) E.Horak. No Tasmanian material has yet been found which can be assigned to *C. apricosa* which has a conical pileus and distinctly ellipsoid spores. The misapplication of *Hygrophorus aurantius* Murrill by G. Stevenson is covered by Horak (1990), p. 278.

2. *Hygrocybe cheelii* A.M.Young, *Austrobaileya* 5: 547 (1999). *Type*: New South Wales. Gladesville, 17.vi.1916, *J.B.Cleland*, s.n. (holotype AD *3418*).

Cantharellus lilacinus Cleland & Cheel, *Trans. & Proc. Roy. Soc. S. Australia* 43: 271 (1919). *Type*: New South Wales. Gladesville. 17.vi.1916. *J.B.Cleland, s.n.* (holotype AD *3418*). *Camarophyllus lilacinus* (Cleland & Cheel) E.Horak, *New Zealand J. Bot.* 28: 203 (1990); *non Hygrocybe lilacina* (C.Laest. *ex* P.Karst.) M.Moser, *Die Röhrlinge und Blätterpilze (Agaricales)* 3 ed., 64 (1967).

Illustrations: Young (1999), p. 547; Willis (1963), plate 9, fig.1 as *Cantharellus lilacinus*; Cleland & Cheel (1919), Plate 29, fig.1.

Habitat and distribtuion: gregarious on soil amongst leaf litter or moss in cool temperate rainforest; occasionally in pasture grass. *Hygrocybe cheelii* is widespread and frequent in southern and central Tasmania.

Material examined: Sandspit Forest Reserve nr Wielangta, 27.ix.1997, *G.Gates (hb. Mills 1595*; HO *508608*); Growlingswallet nr Mt Field, 1.x.1998, *A.K.Mills (hb. Mills 1596*; HO *508609*); Growlingswallet nr Mt Field, 1.x.1998, *A.K.Mills (hb. Mills 1597*; HO *508610*); Marion Bay nr Dunally, 5.vi.1999, *G.Gates (hb. Mills 1649*; HO *508615*; MEL *2087782*); Jacksons Bend, 14.x.1998, *G.Gates (hb. Mills 1602*; HO *508571*).

Notes: These are the first collections from Tasmania assigned to this taxon. The spores of these collections measure (6.5–)8–10(–10.5) × (5.5–)6–6.5(–8) µm, mean range 8.3–9.1 × 6.3–6.6 µm, Q: 1.1–1.6(–1.8), range of mean Q: 1.31–1.45 and are somewhat larger than those of the holotype which measure 6–8.5 × 4.5–6 µm, mean 7.2 × 5 µm, Q: 1.2–1.7, mean Q: 1.43 although they have more or less the same dimension ratio as indicated by the similarity of the two sets of Q measurements. The basidia have no significant dimensional differences. It is interesting to note however, that a collection of *H. cheelii* from Victoria (Young 2000c) has spores measuring 7 –10 × 4.5–5.5(–6.5) µm, mean 8.4 × 5.4 µm, Q: 1.3–1.8, mean Q: 1.56. One possible hypothesis is that spore size increases with latitude, but more collections of the taxon will be required to confirm this. It may also be that the larger spores found in the Victorian and Tasmanian collections are 'normal' while the smaller spores of the holotype represent a taxon variety with unusually small spores.

Collection *hb. Mills 1649* is of interest as it contains a collection in which most of the basidia are 2-spored and only very occasional basidia are 1-, 3- or 4-spored. Clamps are absent or extremely rare throughout the basidiome and only a single clamp connection was sighted in cuticular tissues during the examination. Similar clampless basidiomes where the basidia are predominantly 2-spored are well known in the Hygrophoraceae (Boertmann 1995; Young 2000a).

3. *Hygrocybe reesiae* A.M.Young *in* Young & Wood, *Austral. Syst. Bot.* 10: 923 (1997). *Type*: New South Wales. Lane Cove Bushland Park, 17.vi.1990, *R.Kearney & B.Rees s.n.* (holotype UNSW *90/205*).

Illustration: Young & Wood (1997), p. 924.

Habitat and distribution: gregarious on leaf mould in cool temperate rainforest; frequently amongst moss or litter. The species is frequent in southern and central Tasmania.

Material: Arve Loop Rd nr Geeveston, 11.v.1998, *B.Rees* (*hb. Young 2016*; BRI); Mt Field, 24.iv.1997, *A.K.Mills (hb. Mills 1456*; HO *508586*); Mt Field, 24.iv.1997, *A.K.Mills (hb. Mills 1460*; HO *508589*); *Location and date unknown*; *A.J.Monks* (ZT *4574*; BRI); Mt Field, 16.iv.1991, *E.Horak* (ZT *4350*; BRI).

Remarks: *Hygrocybe reesiae* is at first bright lilac with a hygrophanous pileus. As drying proceeds, the lilac colour becomes fainter and the pileus surface changes to a lilac tinted buff. There is never a distinctly yellow stem base as occurs in *H. cheelii*. Some Tasmanian collections have exhibited a tendency to produce more ellipsoid spores with very few subglobose spores. As a result, spore measurements remain very similar to those of the holotype, but Q's are greater (1.4–1.7, mean 1.48) when compared to those of the holotype (1.1–1.5, mean 1.3). There are no other major differences. There is no doubt that *H. reesiae* is very closely related to the South American taxon *Camarophyllus adonis*, and both taxa almost certainly have a common ancestry. Basidiomes of *C. adonis* have spores which measure 6–9 × 4.5–6.5 µm and basidia which measure 40–60 × 5–7 µm (Horak 1979) and these dimensions are almost identical to those found in basidiomes of *H. reesiae*. *Camarophyllus adonis* has a robust basidiome, branching lamellae which are at most lilac tinted and a stipe which is whitish, yellowish or earthy brown; basidiomes of *H. reesiae* are more slender, have simple lamellae which are bright lilac or violet and violet coloured stipes which dry slowly to buff. Future genetic analysis may show that *H. reesiae* is the Australian variant of *C. adonis* but until this is done, the geographical and

macrocharacter differences of the two taxa are considered sufficient reasons to maintain the separation of the two species.

4. *Hygrocybe rodwayi* (Massee) A.M.Young *in* Young & Wood, *Austral. Syst. Bot.* 10: 923 (1997); *Hygrophorus rodwayi* Massee, *Bull. Misc. Inf. Kew* 1899: 178 (1899). *Camarophyllus rodwayi* (Massee) A.J.Monks & A.K.Mills *in* Banks *et al.* (eds), *Aspects of Tasmanian Botany – A Tribute to Winifred Curtis* 13 (1991). *Type*: Tasmania. Kingston Rd (nr Hobart), undated, *L.Rodway 137* (holotype K).

Illustrations: Fuhrer & Robinson (1992), p. 39; Young & Wood (1997), p. 925.

Habitat and distribution: gregarious to caespitose amongst moss or on soil amongst litter in cool temperate rainforest. *Hygrocybe rodwayi* is common in southern and central Tasmania.

Material: Little Florentine River, 13.v.1998, *A.M.Young (hb. Young 2023*; BRI); Johns Rd nr Geeveston, 19.v.1998, *A.M.Young (hb. Young 2042*; BRI); Johns Road nr Geeveston, 19.v.1998, *A.M.Young (hb. Young 2043*; MEL *2087776*); Little Florentine River, 26.v.1999, *A.M.Young (hb. Young 2268*; MEL *2087766*); Five Road, 24.iv.1997, *A.K.Mills (hb. Mills 1451*; HO *508572*); Mt Field, 24.iv.1997, *A.K.Mills (hb. Mills 1454*; HO *508574*); Johns Road nr Geeveston, 24.ix.1997, *A.K.Mills (hb. Mills 1471*; HO *508575*); Johns Road nr Geeveston, 24.ix.1997, *A.K.Mills (hb. Mills 1472*; HO *508576*); Johns Road nr Geeveston, 24.ix.1997, *A.K.Mills (hb. Mills 1473*; HO *508577*; MEL *2087783*); Johns Road nr Geeveston, 24.ix.1997, *A.K.Mills (hb. Mills 1477*; HO *508578*); Arve Loop Rd nr Geeveston , 11.v.1998, *A.K.Mills (hb. Mills 1535*; HO *508580*); Sandspit Forest Reserve nr Wielangta, 3.vi.1998, *A.K.Mills (hb. Mills 1553*; HO *508582*); Sandspit Forest Reserve nr Wielangta, 3.vi.1998, *A.K.Mills (hb. Mills 1554*; HO *508583*); Rutherford Rd nr Geeveston, 18.vi.1998, *A.K.Mills (hb. Mills 1570*; HO *508570*); Location and date unknown, *A.J.Monks* (ZT *4575*; BRI).

Remarks: The decurrent lamellae and the cream discolouration at the centre of the otherwise white pileus are characteristic of this taxon. Occasional large basidiomes (pilei approaching 40 mm diameter) may be encountered, but all other characters remain constant. Microscopically, the small, subglobose spores measuring (4.5–)5–7(–7.5) × 4.5–5.5(–6) µm are very distinctive. Macroscopically, *H. rodwayi* could be confused with *H. virginea* (Wulfen: Fr.) P.D.Orton & Watling, but the latter species is easily separated microscopically because it has larger, oblong to ellipsoid spores measuring 7.0–12.5 × 3.5–7.5 µm.

Subgenus 2 *Hygrocybe*

Basidiome frequently vividly coloured (red, orange, yellow); *pileus* often conical; *lamellae* free, adnexed or narrowly adnate; *hymenophoral trama* strictly regular, composed of very long (500–3000 µm), tubular, aseptate elements with tapered ends; *cystidia* sometimes present; *clamp connections* generally present throughout the basidiome.

One species in Tasmania.

5. *Hygrocybe astatogala* (R.Heim) Heinem., *Bull. Jard. Bot. État* 33: 436 (1963); *Bertrandia astatogala* (R.Heim) R.Heim, *Rev. Mycol.* 31: 155 (1966). Type: Madagascar. (holotype P, n.v.)

Illustrations: Fuhrer & Robinson (1992), p. 8; Young & Wood (1997), p. 933.

Pileus conical, often red or yellow but rapidly becoming black; all tissues rapidly blackening on bruising and exuding a pale, watery yellow fluid if cut, pileal and stipe surfaces covered in abundant, blackish fibrils.

Habitat and distribution: solitary, gregarious or sometimes in troops amongst leaf litter, moss or directly on soil in cool temperate rainforest; often in very sheltered locations. The species is widely distributed in central, northern and western Tasmania.

Material: Arve Loop Rd nr Geeveston, 27.iv.1998, *A.M.Young (hb Young 1953*; BRI); Mt Field, 30.iv.1998, *A.M.Young (hb Young 1981*; BRI); Tahune, 17.v.1999, *A.M.Young (hb Young 2214;* MEL *2087775*); Tasman Pen., 25.v.1999, *A.M.Young* (*hb Young 2252*; MEL *2087768*); Fire Rd nr Geeveston, 24.iv.1997, *A.K.Mills (hb Mills 1452*; HO *508573*); Johns Rd nr Geeveston, *A.K.Mills (hb Mills 1478*; HO *508579*); Sandspit Forest Reserve nr Wielangta, 3.vi.1998, *A.K.Mills (hb Mills. 1552*; HO *508581*); Franklin R., 13.iv.1991, *E.Horak (*ZT *4323*; BRI); Collinsvale, 3.iv.1991, *E.Horak (*ZT *4806*; BRI).

Remarks: *Hygrocybe astatogala* is widespread and often abundant in Tasmania; troops of 20–30 basidiomes are frequently encountered. Most collections are from amongst leaf litter rather than moss. Although juvenile basidiomes are brilliant red, mature basidiomes are often jet black except for the yellow lamellae. Monks (1989) described Tasmanian variants of *H. astatogala* which differed only in that they had ellipsoid spores [8.5–10.5 × 5.5–7 µm, mean 9.5 × 6.2 µm, mean Q: 1.47] rather than the usual sub-globose spores [7.5–10 × 6–8.5 µm, mean 8.7 × 6.8 µm, Q: 1.0–1.6, mean Q: 1.28]. Two Mills collections (*hb. Mills 1452* and *hb. Mills 1478*) confirm Monks' findings because both of these collections have ellipsoidal spores (many with darker contents) measuring 7–10 × 5.5–6.5 µm, mean 8.8 × 6.3 µm, Q: (1.1–)1.2–1.6, mean Q: 1.39; all other characters of these basidiomes with ellipsoidal spores conform to those of the basidiomes with subglobose spores. Monks also suggested that the difference in spore shape may be a function of the substrate and that moss/litter/peat substrates yield basidiomes with sub-globose spores while substrates derived at least in part from siliceous rocks yield basdiomes with ellipsoid spores. It is not known if the Tasmanian collections detailed above conform to Monks' hypothesis.

Subgenus 3 *Pseudohygrocybe* Bon, *Doc. Mycol.* 24: 42 (1976). *Typical species*: *Hygrocybe coccinea* (Schaeff.: Fr.) P.Kumm.

Basidiome variously coloured often brightly (red, orange, yellow, green, lilac); *pileus* conical, convex or umbilicate; *lamellae* narrowly adnate to decurrent; *cystidia* sometimes present as cheilocystidia, rarely as pseudo-pleurocystidia; *hymenophoral trama* regular, subregular to slightly irregular, composed of short, cylindrical to inflated elements 20–300 µm long (rarely up to 700 µm); clamp connections generally present throughout the basidiome.

6. *Hygrocybe anomala* A.M.Young, in Young & Wood *Austral. Syst. Bot.* 10: 919 (1997). *Type*: New South Wales, Blackheath, 23.vi.1983, *A.E.Wood* s.n. (holotype UNSW *83/991*).

1. Pileus at first viscid; without lilac tints on pileus or lamellae .....6a. var. ***anomala***
1. Pileus always dry; lilac tints present on either or both pileus and lamellae .....
.....6b.var. ***ianthinomarginata***

6a. *Hygrocybe anomala* A.M.Young var. ***anomala***
Illustration: Young & Wood (1997), p. 920.

Habitat and distribution: gregarious amongst moss in cool, temperate rainforest; variety *anomala* is known from locations in southern, central and north-western Tasmania.

Material: Julius River nr Smithton, 6.vi.1998, *A.M.Young (hb. Young 1987*; BRI); Arve Loop Rd nr Geeveston, 11.v.1998, *A.M.Young* (*hb. Young 2020*; BRI); Little Florentine River, 26.v.1999, *A.M.Young* (*hb. Young 2263*; BRI); Arve Loop Rd nr Geeveston, 27.iv.1998, *A.M.Young* (*hb. Young 1963*; MEL *2087779*); Arve Loop Rd nr Geeveston, 17.v.1999, *A.K.Mills (hb. Young 2222*; MEL

2087774); Arve Loop Rd nr Geeveston, 17.v.1999, *A.K.Mills (hb. Young 2224*; MEL *2087773*); Little Florentine River, 26.v.1999, *A.M.Young* (*hb. Young 2264*; MEL *2087767*).

Remarks: *Hygrocybe anomala* var. *anomala* has both a viscid pileus and stipe and there are no lavender/lilac tints on either pileus or lamellae. It appears to be widespread and fairly common in Tasmania.

6b. *Hygrocybe anomala* A.M.Young var. ***ianthinomarginata***, A.M.Young, *Austrobaileya* 5: 551 (1999). *Type*: New South Wales, Lane Cove Bushland Park, 13.vi.1998, *R. & E.Kearney & A.M.Young (hb. Young 2111*) (holotype DAR *73918*).

Illustration: Young (1999), p.552.

Habitat and distribution: gregarious amongst moss or humus in cool temperate rainforest: variety *ianthinomarginata* is known from southern and north-western Tasmania.

Material: Julius River, 6.v.1998, *A.M.Young (hb. Young 1986*; BRI); Johns Road, 19.v.1998, *A.M.Young (hb. Young 2048*; BRI); Franklin River, 21.v.1999, *A.M.Young (hb. Young 2247*; BRI); Franklin R., 7.v.1998, *A.M.Young (hb. Young 2002*; MEL *2087778*); Growlingswallet nr Mt Field, 19.v.1999, *G.Gates* (*hb. Young 2231*; MEL *2087771*).

Remarks: Tasmanian material identified as this variety always has a dry pileus and stipe. Microscopic examination shows that both pileus and stipe have a simple cutis and never display the ixocutis present in var. *anomala*. The type description states that the pileus has a lilac margin and may be slightly umbonate, whereas Tasmanian material is often distinctly umbonate and the lilac marginal tints are weak or lacking. However, the lamellae are always distinctly lilac tinted and microscopic examination always shows the presence of spinose spores. Despite the slight differences, the Tasmanian material is confidently assigned to var. *ianthinomarginata*.

7. *Hygrocybe cantharellus* (Schwein.) Murrill, (as *Hydrocybe*), *Mycologia* 3: 196 (1911); *Agaricus cantharellus* Schwein., *Schriften Naturf. Ges. Leipzig* 1: 88 (1822); *Hygrophorus cantharellus* (Schwein.) Fr., *Epicr. Syst. Mycol.*: 329 (1838). *Type*: none designated.

Illustrations: Boertmann, D (1995): p. 111; Young & Wood (1997), p. 962.

Habitat and distribution: gregarious amongst moss and humus in cool temperate rainforest or gregarious amongst moss in heath. The species appears to be widespread in southern and central Tasmania.

Material: Tahune, 17.v.1999, *A.M.Young & A.K.Mills* (*hb. Young 2213*; BRI); Arve Loop Rd nr Geeveston, 27.iv.1998, *A.M.Young (hb. Young 1968*; MEL *2088590*); Limebay Reserve, 12.v.1999, *A.K.Mills (hb. Young 2209*; MEL *2088608*); Arve Loop Rd nr Geeveston, 17.v.1999, *A.K.Mills* (*hb. Mills 1514*; HO *508596*).

Remarks: Tasmanian material has the usual distinguishing characteristics of this taxon: a dry, red pileus with a finely velvety surface (at least at the centre), bright red, dry stipe, and pale yellow, decurrent lamellae. The basidiomes usually have stipes that are at least 2–3 times longer than the pileus diameter. The species does not appear to be particularly common. *Hygrocybe cantharellus* is one of the very few species of Tasmanian Hygrophoraceae that has been found outside the cool temperate rainforests; there is a single collection (*hb. Young 2209*) from heath.

8. *Hygrocybe chromolimonea* (G.Stev.) T.W.May & A.E.Wood, *Mycotaxon* 54: 147–150 (1995); *Hygrophorus chromolimoneus* G.Stev., *Kew Bull.* 16: 383 (1962). *Gliophorus*

chromolimoneus (G.Stev.) E.Horak, *Beih. Nova Hedwigia* 43: 167 (1973). *Type*: New Zealand. Lake Rotoiti, 16.v.1956, *E.B.Kidson* (*Stevenson 1088*) (holotype K).

Illustrations: Fuhrer and Robinson (1992): p. 41; Young & Wood (1997), p. 964.

Habitat and distribution: gregarious to caespitose amongst moss or humus in cool temperate rainforest; sometimes in association with old tree fern bases. The species is widespread and common.

Material: Arve Loop Rd nr Geeveston, 27.iv.1998, *A.M.Young (hb. Young 1967*; BRI); Milkshake Reserve, 6.v.1998, *A.M.Young (hb. Young 1997*; BRI); Julius R., 6.v.1998. *A.M.Young (hb. Young 1990*; MEL *2088592*); Little Florentine R., 13.v.1998, *A.M.Young (hb. Young 2027*; MEL *2088603*); Growlingswallet nr Mt Field, 19.v.1999, *G.Gates (hb. Young 2232*; MEL *2088611*); Arve Loop Rd nr Geeveston, 17.v.1998, *A.K.Mills* (*hb. Mills 1512*; HO *508594*); Arve Loop Rd nr Geeveston, 17.v.1998, *A.K.Mills* (*hb. Mills 1513*; HO *508595*); Roger River Reserve, 6.v.1998, *A.K.Mills* (*hb. Mills 1520*; HO *508599*); Sandspit Forest Reserve nr Wielangta, 3.vi.1998, *A.K.Mills (hb. Mills 1550*; HO *508605*); Sandspit Forest Reserve, 23.iv.1991, *E.Horak (*ZT *4957*; BRI); Franklin R., 13.iv.1991, *E.Horak (*ZT *4336*; BRI).

Remarks: The viscid and wholly bright yellow basidiomes with convex, umbilicate pilei are very distinctive. The greyish, glutinous margin on each lamella can be seen with the unaided eye but is very easily seen with a ×10 lens. A difficulty when examining herbarium material is that the cheilocystidia often collapse and adhere strongly to each other within the gluten layer but the latter does remain very clearly visible as a translucent region on the lamella margin. Gentle heating in the microscope mountant is often required before the cheilocystidia will separate so that they can be clearly observed.

Tasmanian material of *Hygrocybe chromolimonea* sometimes has slightly larger spores [(8–)8.5–10.5(–12) × 4.5–6.5 µm] than either the holotype (7.5–9.5 × 4.5–6 µm) or typical Australian mainland material [(6.5–)7–9(–11) × 4–6(– 6.5) µm] however because there is so much overlap of spore ranges and all other aspects of the basidiomes remain constant, this difference in spore size is not considered important.

9. *Hygrocybe erythrocrenata* Monks & A.K.Mills, *Mycotaxon* 46: 87 (1993). *Type*: Tasmania. Moonlight Ridge nr Lune River, viii.1989, *A.J.Monks* s.n. (holotype HO *131196*).

Illustration: Mills & Monks (1993), p. 89.

Spores (6–)7–9.5 × 3.7–5.5 µm, mean 7.5 × 4.3 µm, Q: 1.4–2.1, mean Q: 1.73, ellipsoid to subcylindrical, smooth, hyaline, occasionally constricted. *Basidia* 41–59 × 6–8.5 µm, mean 48.4 × 7.2 µm, Q: 5.3–8.8, mean Q: 6.71, 2- or 4-spored, clamped. *Hymenophoral trama* regular to subregular and consisting of hyaline, thin-walled, cylindrical to ellipsoid, inflated elements 29–72 × 5.5–17 µm, clamp connections abundant. *Pileipellis* a cutis of cylindrical, hyaline, septate, thin-walled hyphae 2.5–8.5 µm diam., clamp connections abundant. *Stipitipellis* a cutis of cylindrical, hyaline, septate, thin-walled hyphae 1.5–5 µm diam., clamp connections abundant.

Habitat and distribution: solitary or gregarious on soil, in humus or amongst moss in cool temperate rainforest. The species appears to be widespread and reasonably common.

Material: Arve Loop Rd nr Geeveston, 27.iv.1998, *A.M.Young (hb. Young 1959*; BRI); Little Florentine R., 13.v.1998, *A.M.Young* (*hb. Young 2025*; BRI); Little Florentine R., 13.v.1998, *A.M.Young* (*hb. Young 2032*; MEL *2090262*); Little Florentine R., 13.v.1998, *A.K.Mills & G.Gates* (*hb. Young 2270*; MEL *2090267*); Moonlight Ridge nr Lune River, viii.1989, *A.J.Monks* s.n. (holotype HO *131196*).

Remarks: Re-examination of the holotype has provided the additional microcharacter data listed above. Spore measurements taken from different basidiomes in the holotype demonstrate that otherwise identical basidiomes can produce different sized spores. The

spore sizes obtained from the holotype are a little larger than those of the original description [(5.5–)6–8 × 3.5–5 µm], but these larger spore dimensions correlate almost perfectly with the three collections made of this taxon during 1998.

The protologue of *Hygrocybe erythrocrenata* states that colour variations of the subdecurrent to decurrent lamellae occur, and indicates that such variations may be found within a population of basidiomes occurring in a restricted area (and by implication from the same mycelium). Mills and Monks (1993) stated that lamellae colour variations range from "white or yellowish white to pinkish white (7–10A2) or pallid reddish grey (12B2)". Collections made during 1998 and positively assigned to this taxon have adnate lamellae with at most a decurrent tooth; the lamellae are coloured either dark red (10C7) or violaceous red (12A3–12A4). These collections also produce the violet-magenta spore print described in the protologue, have the same red pileus and stipe and their microcharacters show only the minor variations that can be normally expected within a species. *Hygrocybe erythrocrenata* thus appears to be a taxon which exhibits considerable colour variation of the lamellae. The variants with whitish lamellae appear to produce white spore prints while the dark red to violaceous red lamellae variants produce white spore prints that show the violet-magenta tints when the spores are scraped together into a small mass. All basidiomes have the same colours and surfaces for the pileus and stipe and their microcharacters.

The vivid red stipe reported in this taxon after drying (Mills & Monks 1993) fades with time. Material inspected immediately after the drying process is complete has brownish pilei with intensely carmine-red stipes, however the carmine-red colouration typically slowly disappears and stipes of dried material that is two or three years old are usually only faintly red tinted or brownish. The holotype shows this tendency to fade: eleven years after collection, only a single stipe has any trace of the carmine-red color and the remainder are yellowish brown or have a slight orange tint.

10. *Hygrocybe firma* (Berk. & Broome) Singer, *Sydowia* 11: 355 (1957). *Hygrophorus firmus* Berk. & Broome, *J. Linn. Soc., Bot.* 11: 563 (1871). *Type*: Sri Lanka. Peradeniya, i.1869, *H.K.Thwaites* (*Thwaites 880*) (holotype K, *n.v.*)

Illustrations: Young, Bougher & Robinson (2000), p. 43; Young (2000b), cover photo; Fuhrer & Robinson (1992), p. 44 (as *Hygrocybe procera*).

Habitat and distribution: gregarious to caespitose in moss beds in cool temperate rainforest. The species appears to be common and widespread in central and north-western Tasmania.

Material: Franklin R., 7.v.1998, *A.M.Young (hb. Young 2004*; BRI); Arve Loop Rd nr Geeveston, 17.v.1999, *A.M.Young* (*hb. Young 2221*; BRI); Growlingswallet nr Mt Field, 19.v.1999, *A.M.Young* (*hb. Young 2238*; BRI); Franklin R., 7.v.1998, *A.M.Young* (*hb. Young 2010*; MEL *2090261*); Franklin R., 21.v.1999, *A.M.Young* (*hb. Young 2245*; MEL *2090264*); Tasman Peninsula, 25.v.1999, *A.M.Young* (*hb. Young 2256*; MEL *2090265*); Rutherford Rd nr Geeveston, 18.vi.1998, *A.K.Mills* (*hb. Mills 1580*; HO *509005*); Mt Field, 20.iv.1991, *E.Horak* (ZT *4942*; BRI).

Remarks: *Hygrocybe firma* seems to be common in Tasmanian moss forests and is sometimes mistaken for *Hygrocybe miniata*, which appears to be relatively rare. The two are readily separated microscopically as *H. firma* has dimorphic spores, with the macrospores measuring 11–18 µm in length while *H. miniata* has monomorphic spores measuring 7–9.5(–10) µm in length. Macroscopically, Tasmanian specimens of *H. firma* seem to produce extremely long stipes and the pilei tend to remain hemispheric and umbilicate rather than expanding to the more or less plane pilei with central depressions that are encountered in some of the tropical variants.

Initial observations on lamellae samples suggested both spore and basidia dimorphism. Since a spore print (or similar deposit) may be considered to contain a very high

proportion of mature spores, two samples of stipe surface immediately underneath the lamellae were examined for deposited spores and all spores on each sample were measured. This procedure aimed to maximise a random selection of fully matured spores. A combined total of 85 macrospores and microspores were measured and the results forwarded to Dr David Ratkowsky of the University of Tasmania for statistical analysis (Univariate Procedure - SAS System, SAS Institute 1990). Dr Ratkowsky's analysis confirmed the bimodal distribution and provided further evidence that the Tasmanian populations belong to *H. firma*.

Hygrocybe firma is an exceptionally variable taxon with both yellow and red pilei which may be hemispheric, convex or umbilicate (Corner 1936; Dennis 1970; Pegler 1983, 1986). Corner's seventeen varieties (Corner 1936) have not been widely used and scepticism with respect to the constancy of the varietal characters (Heim 1967) led to his concept of *H. firma* as a variable species. Pegler (1983) used *H. firma* var. *firma* but later (Pegler 1986) preferred to consider the taxon as variable and used only the epithet of *H. firma*. His concept is followed here. Western Australian material considered to be *H. firma* (Young *et al.* 2000) has red, convex to deeply umbilicate pilei which fade to orange or yellow-orange and this agrees wholly with material from the type locality of Sri Lanka (Pegler 1986). The Tasmanian material has brilliant red pilei and stipes and reddish tinted lamellae. This also conforms with known variations of the taxon.

There remains the cluster of taxa reported from New Zealand (Horak 1990) which includes *Hygrocybe firma*, *H. miniceps* (G.Stev.) E.Horak and *H. procera* (G.Stev.) E.Horak, all of which have yellow to red basidiomes, trichoderms on the pilei and large spores. Currently, the last two taxa can be separated from *H. firma* because they are not considered to have dimorphic spores or basidia and *H. procera* is then separated on the basis of its amygdaliform spores compared to the ellipsoidal spores of *H. miniceps*. The two latter taxa are obviously closely related and may be variants of a single taxon. Neither has yet been confirmed for Australia.

11. *Hygrocybe franklinensis* A.M.Young & A.K.Mills, *sp. nov.*

Pileus 9–18(–25) mm, scarlatinus, conicus vel papillato-umbonatus deinde lato-conicus, glaber, viscidus, striatus, ad marginem concolorum vel flavidum. Lamellae adnatae vel subadnatae, aurantiaco-rosae, ad marginem subflavidae. Stipes 25–45 × 2–5 mm, scarlatinus, siccus, laevis, cylindricus, ad basim flavidum. Sporae (7–)8–9.5(–10.5) × 5–6 (–6.5) µm, ovoideae vel ellipsoideae, hyalinae, aliquot subconstrictae. Basidia 38–52 (–60) × 7–9.5(–10.5) µm, 4-spora, fibulata. Cystidia nulla. Trama hymenophoralis regularis vel subregularis, fibulata. Epicutis pilei ixocutem formans. Gregaria in musco sylvestri.

Type: Tasmania. Franklin R., 21.v.1999, *A.M.Young* (*hb. Young 2243*) (holotype HO *508993*)

Pileus 9–18(–25) mm, brilliant red (10A8), acutely conical and usually with a sub-papillate umbo expanding to broad conical, smooth, viscid, striate when moist, margin sometimes concolorous but usually yellow tinted, even or a little ragged occasionally slightly crenulate. *Lamellae* narrowly adnate and then usually with a slight decurrent tooth or ascending-adnate, orange-pink (6A5–8A5), margins are pale yellow (4A6) and even. *Stipe* 25–45 × 2–5 mm, red (10A8) near the pileus but yellow towards the base (3A4), smooth, dry, hollow, cylindrical.

Spores (7–)8–9.5(–10.5) × 5–6 (–6.5) µm, mean 9.0 × 5.6 µm, Q: 1.5–1.8 (–2.0), mean Q: 1.65, ovoid to ellipsoid, hyaline, smooth, occasionally a little constricted. *Basidia* 38–52 (–60) × 7–9.5(–10.5) µm, mean 48.1 × 8.1 µm, Q: 4.3–6.8(–7.2), mean Q: 5.93, 4-spored (occasionally 2-spored), clamped. *Cystidia* absent. *Hymenophoral trama* regular to sub-regular, composed of thin-walled, hyaline, inflated, cylindrical to fusiform or

moniliform elements 25–95 × 7–25 μm, clamp connections abundant. *Pileipellis* an ixocutis composed of repent, cylindrical, septate, thin-walled hyphae, 2–4.5 μm diam., clamp connections abundant. *Stipitipellis* a cutis of repent, cylindrical, thin-walled, septate hyphae 2–5(–7) μm diam., clamp connections abundant. (Fig. 1)

Habitat and distribution: solitary or gregarious amongst moss in cool temperate rainforest. The species appears to be widespread in southern and north-western Tasmania.

Material: Franklin R., 7.v.1998, *A.M.Young* (*hb. Young 2007*; BRI); Arve Loop Rd Harz Mtn, 17.v.1999, *A.M.Young (hb. Young 2228*; BRI); Arve Loop Rd nr Geeveston, 17.v.1999, *A.M.Young* (*hb. Young 2223*; MEL *2090263*); Franklin R., 21.v.1999, *A.M.Young* (*hb. Young 2243*; HO *508993*, holotype).

Remarks: *Hygrocybe franklinensis* is similar to *H. xanthopoda* A.M.Young which also has a scarlet, conical, viscid pileus but differs by having pure yellow, adnexed (occasionally ascending-adnate to very narrowly adnate) lamellae and a yellow to orange-yellow, inflated stipe. Both *H. franklinensis* and *H. xanthopoda* have an ixocutis on the pileus and a dry stipe which separates them from the New Zealand species *H. cavipes* E.Horak which has an ixotrichoderm on the pileus and a viscid stipe.

Etymology: After the type locality of Franklin River, Tasmania.

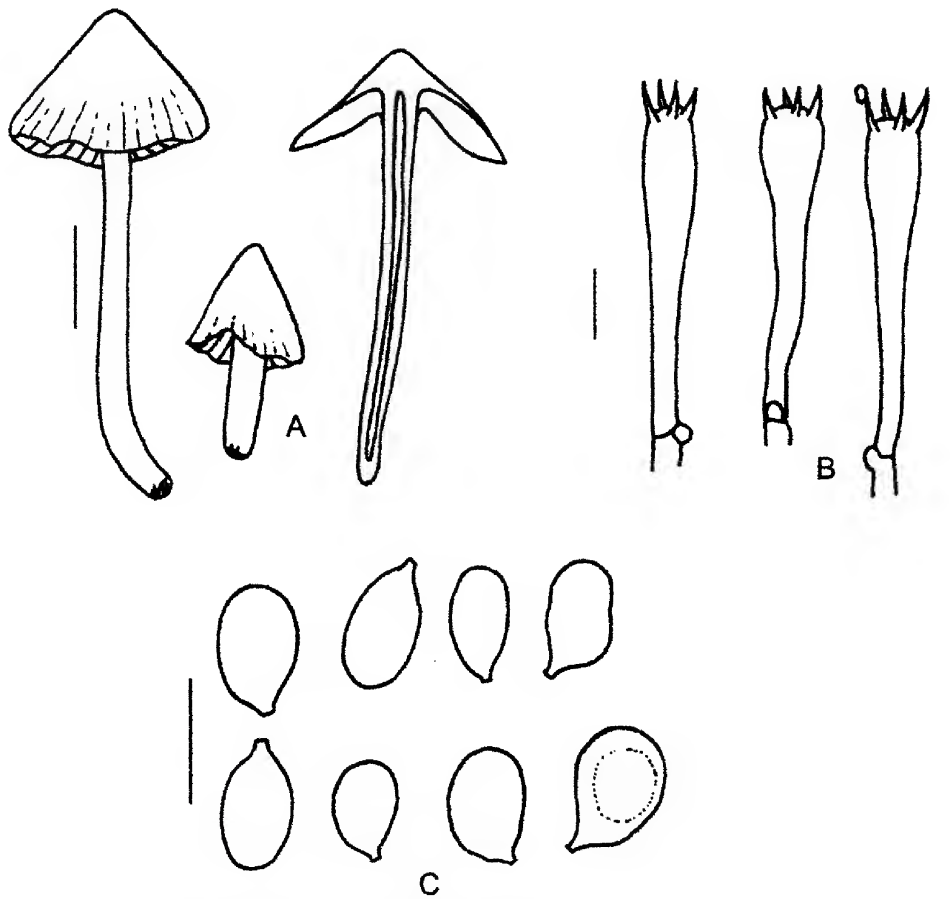

Figure. 1. *Hygrocybe franklinensis* (holotype). **A** habit; **B** basidia; **C** spores. Habit and T/S sketch, bar = 10 mm; microcharacters, bar = 10 μm.

12. *Hygrocybe graminicolor* (E.Horak) T.W.May & A.E.Wood, *Mycotaxon* 54: 147–150 (1995). *Gliophorus graminicolor* E.Horak, *Beih. Nova Hedwigia* 43: 176 (1973). *Type*: New Zealand. Ngahere, 21.iii.1968, *E. Horak* s.n. (holotype PDD *27096*).

Hygrocybe batesii A.M.Young, *Austral. Syst. Bot.* 10: 956 (1997). *Type*: Australia. New South Wales, Monga State Forest, 16.v.1984, *A.E.Wood & N.B.Gartrell* s.n. (holotype UNSW *84/522*).

Gliophorus pallidus E.Horak, *Beih. Nova Hedwigia* 43: 164 (1973). *Type*: New Zealand. Auckland, 27.vi.1968, *E. Horak* s.n. (holotype PDD *27090*).

Illustrations: Fuhrer & Robinson (1992), p. 40; Young & Wood (1997), p. 975 and p. 958 as *H. batesii*.

Habitat and distribution: gregarious to caespitose in humus, litter and moss in cool temperate rainforests. The species appears to be very common and widespread in central and north-western Tasmania and often occurs in large troops.

Material: Arve Loop Rd nr Smithton, 27.iv.1998, *A.M.Young* (*hb. Young 1955*; BRI); Tahune, 27.iv.1998, *A.M.Young* (*hb. Young 1957*; BRI); Tahune, 27.iv.1998, *A.K.Mills* (*hb. Young 1960*; BRI); Tahune, 27.iv.1998, *A.M.Young* (*hb. Young 1962*; BRI); Mt Field, 30.iv.1998, *A.M.Young* (*hb. Young 1973*; BRI); Franklin R., 7.v.1998, *A.M.Young* (*hb. Young 2005*; BRI); Milkshake Reserve nr Smithton, 6.v.1998, *A.M.Young* (*hb. Young 1995*; MEL *2088594*); Lake Chisholm, 6.v.1998, *A.M.Young* (*hb. Young 2000*; MEL *2088595*); Franklin R., 7.v.1998, *A.M.Young* (*hb. Young 2008*; MEL *2088596*); Franklin R., 7.v.1998, *A.M.Young* (*hb. Young 2009*; MEL *2088597*); Arve Loop Rd nr Geeveston, 11.v.1998, *A.M.Young* (*hb. Young 2015*; MEL *2088598*); Tahune, 17.v.1999, *A.M.Young & A.K.Mills* (*hb. Young 2218*; MEL *2088610*); Five Road, 24.iv.1997, *A.K.Mills* (*hb. Mills 1458*; HO *508587*); Five Road, 24.iv.1997, *A.K.Mills* (*hb. Mills 1459*; HO *508588*); Five Road, 24.iv.1997, *A.K.Mills* (*hb. Mills 1461*; HO *508590*); Johns Rd nr Geeveston, 24.ix.1997, *A.K.Mills* (*hb. Mills 1475*; HO *508994*); Arve Loop Rd nr Geeveston, 17.v.1998, *A.K.Mills* (*hb. Mills 1511*; HO *508593*); Arve Loop Rd nr Geeveston, 17.v.1998, *A.K.Mills* (*hb. Mills 1515*; HO *508597*); Arve Loop Rd nr Geeveston, 17.v.1998, *A.K.Mills* (*hb. Mills 1516*; HO *508598*); Franklin R., 7.v.1998, *A.K.Mills* (*hb. Mills 1530*; HO *508600*); Arve Loop Rd nr Geeveston, 11.v.1998, *A.K.Mills* (*hb. Mills 1536*; HO *508601*); Sandspit Forest Reserve nr Wielangta, 3.vi.1997, *A.K.Mills* (*hb. Mills 1551*; HO *508606*); Sandspit Forest Reserve nr Wielangta, 9.vi.1997, *A.K.Mills* (*hb. Mills 1562*; HO *509002*); Jacksons Bend, 14.x.1998, *G.Gates* (*hb. Mills 1605*; HO *508611*); Jacksons Bend, 14.x.1998, *G.Gates* (*hb. Mills 1606*; HO *508612*); Sandspit Forest Reserve, 23.iv.1991, *E.Horak* (ZT *4956*; BRI); Collinsvale, 3.iv.1991, *E.Horak* (ZT *4809*; BRI); Milkshake Reserve, 9.iv.1991, *E.Horak* (ZT *4886*; BRI).

Remarks: The green to brown colour variations of this taxon have already been fully described in Young (1999). *Hygrocybe graminicolor* is often extremely spectacular in the size of its troops amongst moss and 50 or more basidiomes may appear. Occasional variants appear (*hb. Mills 1562*) in which the cheilocystidia are greatly reduced in number. These variants correspond to the normal characteristics in every other way (including spore size 6.5–7.7 × 4–4.5 µm) but cheilocystidia are only occasionally seen and the lamellae margins become fertile. Without the cheilocystidia present, the gluten thread tends to disappear as the basidiomes age.

13. *Hygrocybe irrigata* (Pers.: Fr.) Bon, *Doc. mycol.* 6: 41 (1976). *Agaricus irrigatus* Pers., *Syn. meth. Fung.*: 361 (1801). *Agaricus irrigatus* Pers.: Fr., *Syst. Mycol.* 1: 101 (1821). *Hygrophorus irrigatus* (Pers.: Fr.) Fr., *Epicr. Syst. Mycol.*: 329 (1838). *Type*: none designated.

Illustrations: Boertmann (1995), p. 89; Young, Kearney & Kearney (2001, in edit.)

Habitat and distribution: gregarious amongst moss in cool temperate rainforest. The species is known from central and south-eastern Tasmania.

Material: Little Florentine River, 13.v.1998, *A.M.Young (hb. Young 2030*; MEL *2088605*); Sandspit Forest Reserve nr Wielangta, 1.xii.1998, *G.Gates & D.Ratkowsky* (*hb. Mills 1612*; HO *509012*).

Remarks: In the field, this material resembles an 'all-grey to grey-brown' and extremely glutinous version of *Hygrocybe graminicolor,* however there is never a tint of either yellow or green at any stage on any part of the basidiome and the pilei are more or less conical rather than the convex and usually umbilicate pileus found in *H. graminicolor*. Dried material is greyish to brown and not the brick-pink associated with dried material of *H. graminicolor*. The gluten often has the appearance of a thickly applied layer. The field notes with *hb. Young 2030* recorded that the fresh lamellae margins had a glutinous thread, however, microscopic examination showed no traces of the gluten, no cheilocystidia and fertile lamellae margins. It is assumed that the observed thread was accidental and this assumption is supported by the field notes with collection *hb. Mills 1612* which state that no glutinous thread was present in fresh material.

The material corresponds almost perfectly with *H. irrigata* (Pers.: Fr.) Bon *sensu* Boertmann (1995). The two Tasmanian collections show minor variations of colour and pileus shape (varying from conic to convex) but all are within the range of characters exhibited by European collections. Boertmann (*pers. comm.*) examined material from *hb. Young 2030* and agreed that apart from slightly larger spores (7.5–9.5 × 5–7 µm, mean 8.7 × 6.0 µm, Q: 1.2–1.6, mean Q: 1.5; European material (5–)6.5–8(–9.5) × (3.5–)4.5–5.5(–6) µm, Q: 1.2–2.0, mean Q: 1.4–1.6), the Tasmanian material was well within the range of macrocharacters exhibited by European collections.

14. *Hygrocybe julietae* (G.Stev.) E.Horak, *New Zealand J. Bot.* 9: 431 (1971). *Hygrophorus julietae* G.Stev., *Kew Bull.* 16: 377 (1962). *Type*: New Zealand. Wellington, 1.vii.1949, *G.Stevenson* (*Stevenson 697*) (holotype K).

Illustrations: Horak (1990), p. 269; Stevenson (1963), plate 6 fig. 9.

Pileus 15–18 mm, at first dark greyish yellow (near 4B5) becoming light greyish yellow to clear yellow (near 3A6), convex, dry, margins striate and crenulate or occasionally a little lobed. *Lamellae* decurrent, yellowish grey (4C5/B5) to light yellow (near 3A6), distant, margins even and concolorous. *Stipe* 50–60 × 1.5–2.5 mm, dry, yellow (near 3A6) tapering downwards, solid then becoming narrowly cavernous. *Spore print* white.

Spores 6–8.5 × 3.5–5 µm, mean 6.9 × 3.9 µm, Q: 1.4–2.2, mean Q: 1.80 , smooth, hyaline, cylindrical to ovoid or narrowly ellipsoid, mostly constricted. *Basidia* 33–40 × 6.0–8.5 µm, mean 37 × 7.2 µm, Q: 4.3–6.0, mean Q: 5.13, 4-spored, clamped. *Cystidia* absent. *Hymenophoral trama* regular or occasionally subregular, composed of more or less parallel chains of hyaline, inflated, thin-walled, clamped elements 22–58 × 5–20 µm. *Pileipellis* a dry cutis with some hyphal gelatinisation or a weakly formed ixocutis, hyphae hyaline, thin-walled, cylindrical, septate, 2.5–4.2 µm, clamp connections present (clamp connections sometimes only occasional on upper cutis elements but common on lower cutis elements); lactifers present in subcuticular layers as highly refractive, often contorted or convoluted hyphae, 1–3 µm diameter. *Stipitipellis* a dry cutis with some hyphal gelatinisation or a weakly formed ixocutis with elements that are hyphal, cylindrical, hyaline, thin-walled, clamped, 1–3 µm diameter. (Fig. 2)

Habitat and distribution: gregarious in leaf mould in cool temperate rainforest. The species is known only from the single location in southern Tasmania.

Material: Arve Loop Road nr Geeveston, 11.v.1998, *A.M. Young (hb. Young 2014*; BRI).

Remarks: This taxon with its yellow convex pileus and constricted spores is neither *Hygrocybe blanda* E.Horak with its distinctively conic or campanulate pilei and ellipsoid, non-constricted spores, nor *H. cerinolutea* E.Horak, which does have a convex pileus but has larger spores (8–10 × 5.5–7 µm) without constrictions. The material when collected in the field was quite dry, however microscopic examination clearly demonstrated extensive hyphal gelatinisation on both pileus and stipe together with very firm adherence of

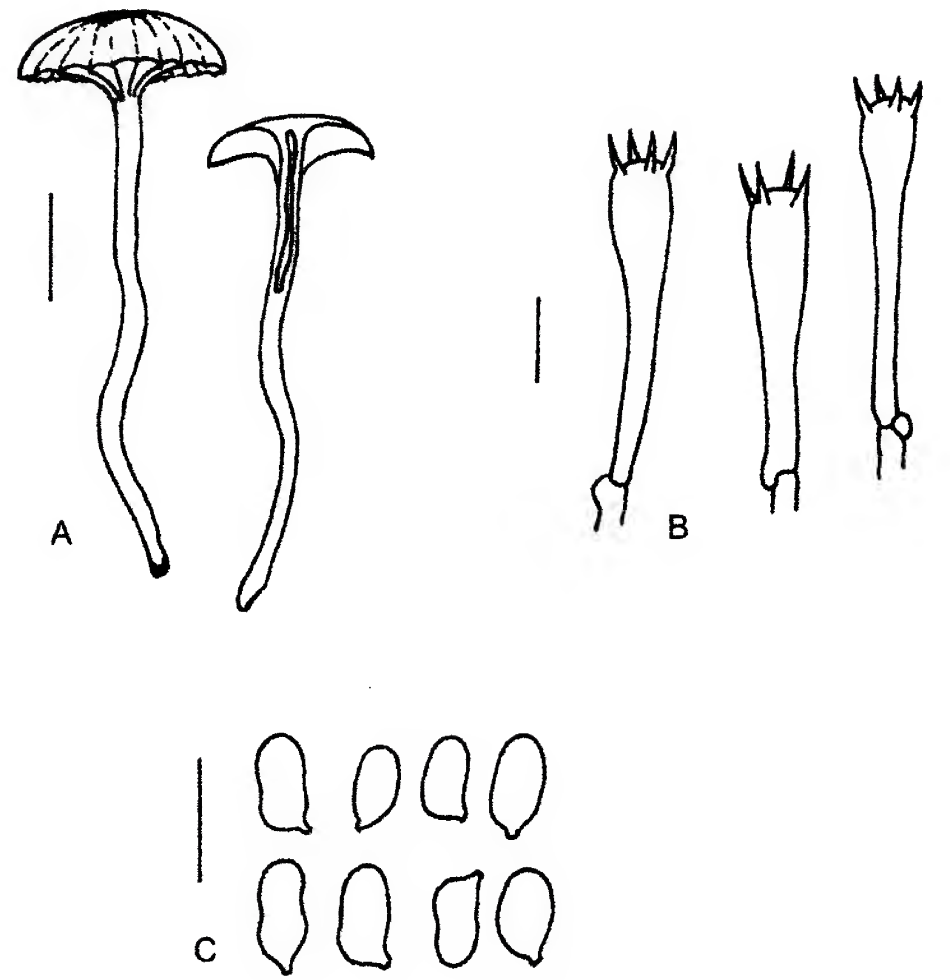

Figure. 2. *Hygrocybe julietae* (*hb. Young 2014*; BRI). **A** habit; **B** basidia; **C** spores. Habit and T/S sketch, bar = 10 mm; microcharacters, bar = 10 µm.

spores to the gelatinised hyphae. Both the protologue and the description of Horak (1990) indicate that *H. julietae* may be either viscid or dry in the field (almost certainly dependent upon local weather conditions); this Tasmanian collection conforms to this characteristic quite well.

15. *Hygrocybe leucogloea* A.M.Young, *Austral. Syst. Bot.* 10: 983 (1997). *Type*: New South Wales. Mt Wilson, 29.iv.1989, *A.E.Wood* s.n. (holotype UNSW 89/87).

Illustration: Young & Wood (1997), p. 984.

Pileus 35 mm, white, more or less convex, viscid. *Lamellae* very narrowly adnate, white, margins even and concolorous. *Stipe* 50 × 8 mm, white, dry, smooth, pith filled.

Spores 6.0–7.7 × 3.7–5.0 µm, mean 6.8 × 4.3 µm, Q: 1.4–1.8 (–1.9), mean Q: 1.57, ellipsoid to narrow ellipsoid, hyaline, smooth. *Basidia* 42–50 × 6–8 µm, mean 45.3 × 6.8 µm, Q: 5.9–7.5, mean Q: 6.69, 4-spored, clamped. *Cystidia* absent. Hymenophoral trama

regular and composed of cylindrical to inflated ellipsoid elements which are hyaline, thin-walled, septate and 20–78 × 3–11 µm, clamp connections abundant. *Pileipellis* a very well developed ixotrichoderm up to 170 µm deep, composed of very loosely interwoven and aerial hyphae embedded in gluten; the ixotrichodermal hyphae are thin-walled, cylindrical, hyaline, 2–3.5 µm diam., clamp connections abundant and usually of medallion form. *Stipitipellis* a cutis (or possibly a very weak ixocutis as some gelatinisation seems to be present) composed of repent, cylindrical, hyaline, thin-walled hyphae 1.5–6 µm diam., clamp connections abundant.

Habitat and distribution: solitary in moss in cool temperate rainforest. The species is known only from the single locality.

Material: Tasman Peninsula, 25.v.1999, *A.K.Mills* (*hb. Young 2251*; MEL *2088614*).

Remarks: The single basidiome in this collection was rather old but there is no doubt as to its identity. *Hygrocybe leucogloea* is the only known Australian taxon within the Hygrocybeae which has pure white basidiomes and an ixotrichoderm on the pileus; these characters are shared by the Tasmanian material. The American species, *Hygrophorus purus* Peck, is similar macroscopically but differs in that it belongs to sub-genus *Humidicutis* and does not have clamp connections anywhere in the basidiome except at the bases of the basidia.

The holotype of *Hygrocybe leucogloea* has spores that measure (6.3–)6.5–7.9(–8.5) × 4.0–5.6 µm, mean 7.2 × 4.8 µm, Q: 1.2–1.7, mean Q: 1.5 while those of the Tasmanian material measure 6–7.7 × 3.7–5.0 µm, mean 6.8 × 4.3 µm, Q: 1.4–1.8 (–1.9), mean Q: 1.57. The differences in dimensions are not considered important at this stage. A Victorian collection (MEL *261035*) contained spores measuring 5.3–7.7 × 4.0–5.3 µm, mean 6.8 × 4.5 µm, Q: 1.3–1.8, mean Q: 1.5. This confirms the slight variations in spore dimensions that are to be found in this taxon (Young 2000a). The Tasmanian material displayed very narrowly adnate lamellae instead of the adnexed lamellae present in the holotype, however this is presently believed to be simply a variation likely to be encountered in future collections of this taxon. If all future Tasmanian collections of this taxon show similar lamellae attachments, a separate Tasmanian variety could be considered.

16. *Hygrocybe lilaceolamellata* (G.Stev.) E.Horak, *New Zealand J. Bot.* 9: 434 (1971). *Hygrophorus lilaceolamellatus* G.Stev., *Kew Bull.* 16: 378 (1963). *Type*: New Zealand. Wellington, 2.vi.1949, *G.Stevenson* (*Stevenson 619*) (holotype K).

Illustrations: Horak (1990), p. 269; Young & Wood (1997), p. 985; Fuhrer & Robinson (1992), p. 42.

Habitat and distribution: gregarious on soil or amongst leaf mould or moss in cool temperate rainforest. The species is widespread and common in southern, central and north-western Tasmania.

Material: Little Florentine R., 13.v.1998, *A.M.Young (hb. Young 2029*; BRI); Growlingswallet nr Mt Field, 19.v.1999, *A.M.Young* (*hb. Young 2226*; BRI); Julius R., 6.v.1998, *A.M.Young (hb. Young 1989*; MEL *2088591*); Arve Loop Rd nr Geeveston, 11.v.1998, *A.M.Young* (*hb. Young 2019*; MEL *2088601*); Little Florentine R., 26.v.1999, *A.K.Mills & A.M.Young (hb. Young 2262*; MEL *2088615*); Roger River Reserve nr Smithton, 6.v.1998, *A.K.Mills* (*hb. Mills 1523*; HO *508996*); Rutherfords Rd nr Geeveston, 18.vi.1998, *A.K.Mills* (*hb. Mills 1578*; HO *509003*).

Remarks: This species is very distinctive with its warm brown pilei and contrasting lilac lamellae. The taxon is very variable with respect to spore size and shape. Mean Q has been found to vary from 1.65–1.97 indicating a variation from predominantly long-ellipsoid spores in the basidiomes of some collections to predominantly cylindrical spores in others. Spore constrictions may be frequent or only occasionally displayed in various collections. Sectioned lamellae from some collections have displayed a weakly

divergent trama and further study may result in the transfer of this species to the genus *Hygrophorus*.

17. *Hygrocybe miniata* (Fr.: Fr.) P.Kumm., *Führ. Pilzk.*: 112 (1871); *Agaricus miniatus* Fr.: Fr., *Syst. Mycol.* 1: 105 (1821); *Hygrophorus miniatus* (Fr.: Fr.) Fr., *Epicr. Syst. Mycol.*: 330 (1838). *Type*: Sweden. Smoland, 21.ix.1980, *M. Moser 80/372* (neotype IB, *n.v.*, designated by Arnolds 1986, p. 148).

Illustrations: Horak (1990), Plate 4, fig. 2; Young & Wood (1997), p. 989.

Habitat and distribution: gregarious on humus amongst moss in cool temperate rainforest. The species is known from only a single collection in south-eastern Tasmania.

Material: Arve Loop Rd nr Geeveston, 27.iv.1998, *A.M.Young* (*hb Young 1964*; BRI).

Remarks: The single known collection suggests that *Hygrocybe miniata* is possibly rare in Tasmania but this should be regarded as very tentative. There may be several reasons for the single collection during the 1998–1999 Tasmanian trips including: the species may fruit abundantly at a different time of year; the most important areas where the fungus occurs have not yet been located; and the years 1998 and 1999 were not suitable years for optimum occurrence of the species. Much more collecting is required over a number of years to assess the species' true abundance and distribution. The basidiomes of this collection strongly resemble those of *H. firma*, but very careful analysis of the spores (including a spore print) has confirmed that they are not dimorphic and that they measure 7.5–9.5 × 4.5–6.5 µm, mean 8.4 × 5.4 µm, Q: 1.3–1.9, mean Q: 1.56. There is no doubt that this collection represents a variant of *H. miniata*.

18. *Hygrocybe pseudograminicolor* A.M.Young, *Austral. Syst. Bot.* 10: 992 (1997). *Type*: Australia. New South Wales. Mt Wilson, 26.iii.1994, *F.Taeker* s.n. (holotype UNSW *94/22*).

Illustration: Young & Wood (1997), p. 994.

Habitat and distribution: gregarious to caespitose amongst leaf litter or in moss of cool temperate rainforests. The species is widespread and common in southern central and north-western Tasmania.

Material: Julius R., 6.v.1998, *A.M.Young (hb. Young 1988*; BRI); Arve Loop Rd nr Geeveston, 11.v.1998,(*hb. Young 2018*; MEL *2088600*); Little Florentine R., 13.v.1998, *A.M.Young (hb. Young 2035*; MEL *2088606*); Roger River Reserve nr Smithton, 6.v.1998, *A.K.Mills (hb. Mills 1518*; HO *508995*); Arve Loop Rd nr Geeveston, 11.v.1998, *A.K.Mills* (*hb. Mills 1543*; HO *508998*); Sandspit Forest Reserve nr Wielangta, 3.vi.1998, *A.K.Mills (hb. Mills 1549*; HO *508999*); Sandspit Forest Reserve nr Wielangta, 3.vi.1997, *A.K.Mills (hb. Mills 1561*; HO *509001*); Sandspit Forest Reserve nr Wielangta, 14.vii.1998, *A.K.Mills (hb. Mills 1587*; HO *509010*); Franklin R., 13.iv.1991, *E.Horak (*ZT *4316*; BRI).

Remarks: Although *Hygrocybe pseudograminicolor* does resemble *H. graminicolor* and both have cheilocystidia, the bright lime green lamellae of the former species serve to distinguish it in the field from the latter taxon which has white or at most green tinted lamellae. The spores of *H. pseudograminicolor* (8.5–10.5 × 5.0–7.7 µm) are larger than those of *H. graminicolor* (5.3–7.3 × 3.3–5 µm). The green pigments of *H. pseudograminicolor* also differ from *H. graminicolor* in that the former species becomes dull green when dried for herbarium storage, but the latter becomes brick-pink. The species is comparatively rare at the type locality but is quite common in Tasmania.

A peculiarity of this taxon is that the lamellar trama alters with maturation of the basidiome. During the early stages of development, the trama is regular but in late maturity it becomes more or less irregular.

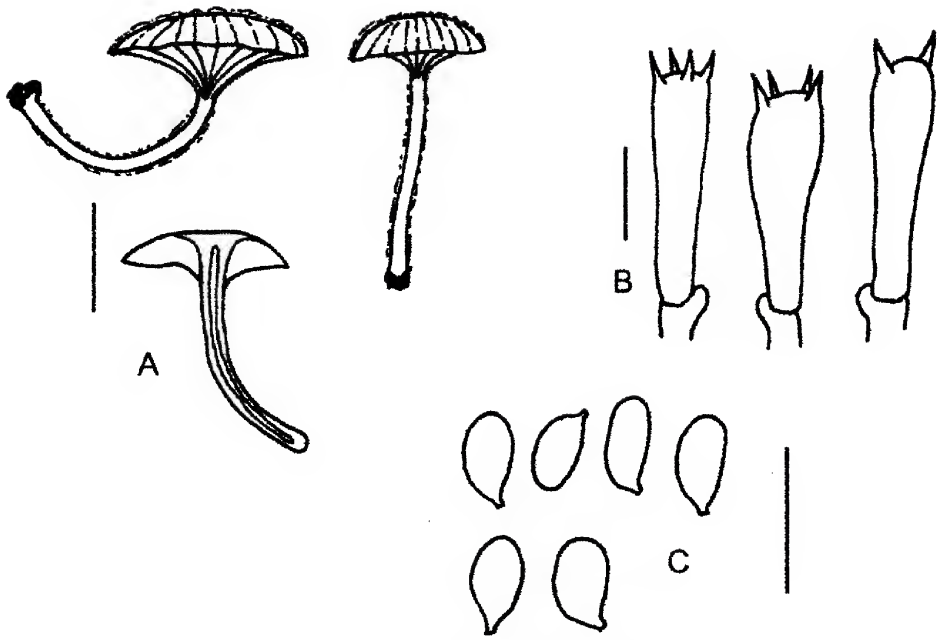

Figure. 3. *Hygrocybe roseoflavida* (holotype). **A** habit; **B** basidia; **C** spores. Habit and T/S sketch, bar = 10 mm; microcharacters, bar = 10 µm.

19. *Hygrocybe roseoflavida* A.M.Young & A.K.Mills, *sp. nov.*

Pileus 9–20 mm, pallido-roseus, convexus, umbilicatus, viscidus, plicato-striatus, ad marginem aequalum vel subcrenulatum. Lamellae decurrentes, pallido-roseae, ad marginem concolores. Stipes 13–20(–40) × 1–2 µm, super pallido-roseo-brunneus, flavidus, viscidus, laevis, cylindricus, cavus, ad basim atroflavidum. Sporae (5–)6–7.5 × 3.5–4.5 µm, ovoideae vel ellipsoideae vel lacrymoideae, hyalinae, nunquam constrictae. Basidia (17–)23–28(–32) × 5.5–7 µm, (2-)4-spora, fibulata. Cystidia nulla. Trama hymenophoralis subregularis vel regularis, fibulata. Epicutis pilei ixocutem formans. Gregaria vel caespitosa in humo sylvestri.

Type: Tasmania. Sandspit Reserve nr Wielangta, 3.vi.1998, *A.K.Mills* (*hb. Mills 1559*) (holotype HO *509000*)

Pileus 9–20 mm, pallid pink (6A2) but often with brownish tints, convex becoming depressed at the centre or umbilicate and finally more or less plane but remaining centrally depressed, plicate-striate but otherwise smooth, viscid to glutinous, margin even to slightly crenulate. *Lamellae* decurrent, pallid pink (13–14A2 or similar to the pileus with brownish tints), distant, without a glutinous thread to the margins, margins even and concolorous. *Stipe* 13–20(–40) × 1–2 mm, pallid pinkish brown (6A2–9A2 or similar to pileus) near the lamellae then becoming pale yellow (2A3) and darker towards the base, viscid to glutinous, cylindrical but sometimes a little inflated at the base, hollow.

Spores (5–)6–7.5 × 3.5–4.5 µm, mean 7.0 × 3.9 µm, Q: 1.4–2.0(–2.2), mean Q: 1.79, ovoid to ellipsoid or lacrymoid, hyaline, smooth, non-constricted. *Basidia* (17–)23–28(–32) × 5.5–7 µm, mean 24.4 ×6.4 µm, Q: (2.5–)3.5–4.4(–4.7), mean Q: 3.82, (2-)4-spored, clamped. Cystidia none. *Hymenophoral trama* regular to sub-regular, composed of hyaline, inflated, thin-walled elements (17–)30–60(–90) × 7–30 µm, clamp connections abundant to occasional. *Pileipellis* an ixocutis of gelatinised, hyaline, thin walled repent hyphae 1.5–7 µm, clamp connections abundant. *Stipitipellis* an ixocutis of

hyaline, repent, septate, thin-walled hyphae 2.5–6.0 µm, clamp connections present on smaller hyphae (< 4 µm diameter) but absent from larger hyphae. (Fig. 3)

Habitat and distribution: gregarious to caespitose on humus rich soil amongst moss and tree fern debris in rainforest gully, often on the bases of old tree ferns in cool temperate rainforest. This species is so far only known from locations in south-eastern Tasmania.

Material: Johns Rd nr Geeveston, 19.v.1998, *A.M.Young* (*hb. Young 2045*; BRI); Mt Wellington, 28.iv.98, *A.M.Young* (*hb. Young 1969*; MEL *2090260*); Tasman Peninsula, 25.v.1999, *A.K.Mills* (*hb. Young 2258*; MEL *2090266*); Sandspit Forest Reserve nr Wielangta, 3.vi.1998, *A.K.Mills* (*hb. Mills 1559*; holotype HO *509000*); Sandspit Forest Reserve nr Wielangta, 14.vii.1998, *A.K.Mills* (*hb. Mills 1585*; HO *509009*).

Remarks: This often tiny, glutinous taxon is rather interesting for its unique colouration of pallid pink or pinkish brown pileus and lamellae but with contrasting yellow stipe. The basidia are also unusual in that they are amongst the smallest in the Hygrophoraceae. No other taxon has these colour characteristics coupled with the very small basidia. It is quite difficult to obtain good representative collections of this species as only a very few basidiomes are normally found at each location.

20. *Hygrocybe stevensoniae* T.W.May & A.E.Wood, *Mycotaxon* 54: 148 (1995). *Hygrophorus viridis* G.Stev., *Kew Bull.* 16: 383 (1963); *Gliophorus viridis* (G.Stev.) E.Horak, *Beih. Nova Hedwigia* 43: 173 (1973); *non Hygrocybe viridis* Capelari & Maziero, *Mycotaxon* 33: 192 (1988). *Type*: New Zealand. Levin, 26.vi.1948, *G. Stevenson 338* (holotype K).

Misapplied: *Hygrophorus psittacinus* sensu Cleland and Cheel (1919) and Willis (1963); *Hygrocybe psittacina sensu* Shepherd and Totterdell (1988).

Illustrations: Fuhrer & Robinson (1992), p. 41; Young & Wood (1997), p. 998.

Habitat and distribution: solitary to gregarious in cool temperate rainforest amongst moss or humus. The species is widely distributed but does not appear to be as common as the very similar glutinous taxon, *Hygrocybe graminicolor*.

Material: Arve Loop Rd nr Geeveston, 11.v.1998, *A.K.Mills (hb. Mills 1540*; HO *508997*); Sandspit Forest Reserve nr Wielangta, 14.vii.1998, *A.K.Mills* (*hb. Mills 1582*; HO *509006*); Sandspit Forest Reserve nr Wielangta, 14.vii.1998, *A.K.Mills* (*hb. Mills 1583*; HO *509007*); Jacksons Bend, 14.x.1998, *A.K.Mills (hb. Mills 1601*; HO *509011*).

Remarks: This taxon is presently accepted as occurring in Tasmania, but material from both Australia and New Zealand requires futher study. The four collections cited here exhibit macrocharacters consistent with the holotype as follows: all display glutinous basidiomes with a well developed ixotrichoderm on the pileus, the lamellae have fertile margins (there is no glutinous thread with embedded hyphal cheilocystidia) and the lamellae are adnate or at most have a decurrent tooth. The spores in all four collections are ellipsoidal and measure 7–9(–10) × 4–5.5(–6) µm, means 7.3–8.0 × 4.4–4.9 µm, Q: 1.4–1.8, mean Q: 1.62 –1.66. This also compares quite well with the holotype which has the same macrocharacters and in which the spores measure 6.5–9(–10) × 4–6.5 µm, mean 8.0 × 4.9 µm, Q: 1.4–1.8 (–2.1), mean Q: 1.63.

The basidial lengths of Tasmanian material [42–64(–70) × 5–8(–9) µm, mean 47.6 –57.5 × 6.8 –7.8 µm, Q: 5.7–9.6, mean Q: 6.98 –7.66] differ consistently from the holotype collection which contains basidia measuring 35–45 × 6–7.5 µm, mean 38.9 × 6.1 µm, Q: 4.7–7.0(–9), mean Q: 6.35. The larger basidia found in Tasmanian collections are also known from several collections accepted as *Hygrocybe stevensoniae* from the Blue Mountains, NSW which had basidia measuring 42–56 × 5–8 µm, mean 46.8–51.8 × 6.8–7.5 µm, Q: 5.4–10, mean Q: 6.21–7.12. These collections had basidiomes which exhibited the usual macrocharacters of the species and which had spores agreeing with

the dimension range exhibited by the spores of the holotype. The longer basidia of the Tasmanian material are therefore not considered to be important.

Two Tasmanian collections (*hb. Mills 1582, 1583*) showed a colour variations in which pinkish tones predominated. Whether this was due to rain wash, bacterial infection or genetic variation is unknown, however all four collections cited here dried to the usual brick-pink colour exhibited by herbarium material of this taxon.

Subgenus 4 *Humidicutis* Singer, *Sydowia* 2: 28 (1948). *Typical species*: *Hygrophorus marginatus* Peck.

Basidiome variously coloured white, pink, dull orange, yellow, or lilac; *pileus* usually conical becoming umbonate or plane and frequently splitting radially; *lamellae* narrowly adnate, adnexed or more or less free; *cystidia* absent; *hymenophoral trama* regular, composed of short, cylindrical to inflated (often moniliform) elements 20–300 µm long; *clamp connections* absent throughout the basidiome except at the bases of the basidia and then frequently of medallion form.

21. *Hygrocybe lewelliniae* (Kalchbr.) Brittleb. *ex* A.M.Young in Young & Wood, *Austral. Syst. Bot.* 10: 1011 (1997); *Hygrophorus lewelliniae* Kalchbr., *Proc. Linn. Soc. New South Wales* 7: 105 (1882). *Type*: Victoria. Western Port, 14.vi.1880, *M.M.R.Lewellin* (holotype Rare Book Mss A11, MEL).

Uncertain recombination: Brittlebank, *Cat. Austral. Fungi* 181 (1940).

Illustrations: Willis (1957); Cole, Fuhrer & Holland (1978), plate 3.

Habitat and distribution: often solitary but occasionally gregarious, usually amongst moss but occasionally in humus in cool temperate rainforest. *Hygrocybe lewelliniae* is common and widespread in southern, central and north-western Tasmania.

Material: Arve Loop Rd nr Geeveston, 27.iv.1998, *A.M.Young* (*hb. Young 1965*; BRI); Mt Field, 30.iv.1998, *A.M.Young* (*hb. Young 1976*; BRI); Lake Chisholm, 6.v.1998, *A.M.Young* (*hb. Young 1999*; BRI; MICH *28502*); Franklin R., 7.v.1998, *A.M.Young* (*hb. Young 2003*; BRI); Little Florentine R., 13.v.1998, *A.M.Young* (*hb. Young 2021*; BRI); Franklin R., 21.v.1999, *A.M.Young* (*hb. Young 2246*; BRI); Arve Loop Rd nr Geeveston, 11.v.1998, *A.M.Young* (*hb. Young 2017*; MEL *2088599*); Little Florentine R., 13.v.1998, *A.M.Young* (*hb. Young 2022*; MEL *2088602*); Johns Rd nr Geeveston, 19.v.1998, *A.M.Young* (*hb. Young 2047*; MEL *2088607*); Tahune, 17.v.1999, *A.M.Young* (*hb. Young 2212*; MEL *2088609*); Franklin R., 21.v.1999, *A.M.Young* (*hb. Young 2242*; MEL *2088612*); Rutherfords Rd nr Geeveston, 18.vi.1998, *A.K.Mills* (*hb. Mills 1579*; HO *509004*).

Remarks: Colour variations of *Hygrocybe lewelliniae* include very pale lilac to nearly deep violet but all collections have shown the same microscopic characters as those of the holotype. Macroscopically, all collections have displayed the conical to umbonate, dry pilei with radial splitting that creates a medial split along the trama in the lamella directly beneath the split in the pileus so that each lamella half remains attached both to the pileus and along the lamella margin. The recombination by Brittlebank (1940) remains doubtful due to uncertainties with respect to effective means of publication and so the paper by Young and Wood (1997) is used to validate Brittlebank's recombination.

22. *Hygrocybe mavis* (G.Stev.) E.Horak, *New Zealand J. Bot.* 9: 434 (1971); *Hygrophorus mavis* G.Stev., *Kew Bull.* 16: 377 (1962). *Type*: New Zealand. Levin, 18.vi.1949, *G. Stevenson*, *Stevenson 654* (holotype K).

Misappl.: *Hygrophorus purus* Peck, sensu E.Horak, *New Zealand J. Bot.* 28: 294 (1990).

Illustration: Young & Wood (1997), p. 1016.

Habitat and distribution: generally solitary but occasionally gregarious and occurring in moss or on soil or amongst humus in cool temperate rainforest. This species is frequent and widespread in southern, central and north-western Tasmania.

Material: Mt Field, 30.iv.1998, *A.M.Young* (*hb. Young 1971*; BRI); Lake Chisholm, 6.v.1998, *A.M.Young* (*hb. Young 1998*; BRI); Johns Rd nr Geeveston, 19.v.1998, *A.M.Young* (*hb. Young 2044*; BRI); Little Florentine R., 26.v.1999, *A.M.Young* (*hb. Young 2265*; BRI); Milkshake Res., 6.v.1998, *A.M.Young* (*hb. Young 1994*; MEL *2088593*); Little Florentine R., 13.v.1998, *A.M.Young* (*hb. Young 2028*; MEL *2088604*); Tasman Peninsula, *A.K.Mills & A.M.Young* (*hb. Young 2249*; MEL *2088613*); Sandspit Forest Res. nr Wielangta, 14.vii.1998, *A.K.Mills* (*hb. Mills 1584*; HO *509008*); Julius R nr Smithton, 9.iv.1991, *E.Horak* (ZT *4854*; BRI).

Remarks: *Hygrocybe mavis* shares with *H. lewelliniae* the remarkable characteristic in which there is combined splitting of both the pileus and lamellae. Whether *H. mavis* is the white variety of *H. lewelliniae* remains to be resolved. The characters of Tasmanian material conform well with those of the holotype.

Genus 2. *Camarophyllopsis* Herink, *Sborn. Severoesk. Mus., PÍir. Vdy* 1: 61 (1958). *Typical species*: *Camarophyllopsis schulzeri* (Bres.) Herink.

Basidiome thin to fleshy, small, dull coloured in grey to ochre or brown; *pileus* convex to umbilicate, dry and often hygrophanous; *lamellae* distant, broadly adnate to arcuate or decurrent; universal veil absent; *stipe* dry, often with small dots or pruinose punctate; spore print white. *Spores* hyaline, smooth, non-amyloid, subglobose to broadly ellipsoid, small (up to 7 µm long); *basidia* narrowly clavate, 20–70 × 4.5–8.5 µm, Q: 4.5–10.0, mostly 4–spored; cystidia absent or inconspicuous; *hymenophoral trama* regular to subregular and composed of short elements up to 170 µm long; *pileipellis* an hymeniderm; *clamp connections* present or absent; development monovelangiocarpic and stipiticarpic. Solitary to subgregarious, terrestrial in forests or open sites, apparently saprophytic. Mostly in temperate North America, Asia and Europe, but also known from subtropical South America and Asia.

23. *Camarophyllopsis kearneyi* A.M.Young, *Austrobaileya* 5: 562 (1999). *Type*: New South Wales. Lane Cove Bushland Park, 13.vi.1998, *R. & E.Kearney* s.n. (holotype DAR *73919*).

Illustration: Young (1999), p. 563.

Habitat and distribution: gregarious to caespitose on soil or deep humus or amongst moss in very sheltered parts of cool temperate rainforest. The two Tasmanian collections come from nearby locations in the south east region.

Material: Growlingswallet nr Mt Field, 19.v.1999, *A.M.Young (hb. Young 2236*; MEL *2089735*); Little Florentine R., 26.v.1999, *A.M.Young (hb. Young 2269*; MEL *2089736*).

Remarks: These two collections conform macroscopically extremely well to the type collection from Lane Cove Bushland Park. Both Tasmanian collections were made in very sheltered locations and the material in *hb. Young 2269* was collected under old tree ferns in very dense shade. The Growlingswallet collection consisted of basidiomes growing gregariously but the second collection had a number of basidiomes additionally displaying a caespitose habit. Spore sizes from the two collections were 4.5–6 × 4.5–5.5 µm, mean 5.4 × 5.2 µm, Q: 0.9–1.2, mean Q: 1.05, and 4.5–6.5 × 4–5.5 µm, mean 5.2 × 4.7 µm, Q: 1.0–1.3, mean Q: 1.10 respectively. This compares well with the holotype which has spores measuring (4 –)4.3–5.7 × 4.0–5.3(–5.7) µm, mean 4.9 × 4.6 µm, Q: 1.0–1.2(–1.3), mean Q: 1.1. The basidial ranges similarly overlap and variations are not considered important.

The prescence and structure of cheilocystidia reported from the holotype appears to vary and is now in need of further clarification. At the time of publication, only material collected from the type location was known and possible character ranges were therefore limited to those exhibited by the holotype. The two Tasmanian collections do contain lamellae elements that appear to be identical to the cheilocystidia of the holotype, however these elements may be partially or wholly hidden within the marginal structure of the lamellae. The Tasmanian collections indicate that this type of variation may be normal for the species and is now being encountered for the first time. In addition, the cheilocystidia often resemble immature basidia/basidioles and the 'fine line' between cheilocystidia and immature reproductive elements is now being considered. Further mature and immature collections of this taxon are required to determine whether the cheilocystidia reported from the holotype are either a variable character or an artifact of the basidiome age/maturity. *Hygrocybe miniata* (Arnolds 1990; Boertmann 1995) is another taxon in which various collections may or may not exhibit the presence of cheilocystidial elements.

TRIBE 2. HYGROPHOREAE P. Henn. *in* Engl. & Prantl, *Nat. Pflanzenfam.* 1: 209 (1898). *emend.* Kühner in *Bull. mens. Soc. linn. Lyon* 48: 617 (1979). *Type genus*: *Hygrophorus* Fr., *Gen. Hymenomyc.*: 8 (1836).

Hymenophoral trama divergent; forming ectomycorrhizae.

Genus 1. *Hygrophorus* Fr., *Gen. Hymenomyc.* 8 (1836). *Typical species*: *Hygrophorus eburneus* (Bull.: Fr.) Fr.

Basidiome tricholomatoid to omphaloid, fleshy to thin, small to large; *pileus* variously coloured but usually of dull colours, not hygrophanous, mostly viscid to glutinous; *lamellae* spaced to distant, broadly adnate to decurrent, thick, waxy; *universal veil* often present and glutinous; *partial veil* sometimes present; *stipe* often glutinous or viscid, frequently with small dots punctate at the apex; *spore print* white. *Spores* hyaline, smooth, non-amyloid, *basidia* narrowly clavate, 30–90 × 6–15 µm, Q: 4.5–9.0; *cystidia* absent or inconspicuous; *hymenophoral trama* divergent from a central line and made of short elements up to 200 µm long; *pileipellis* mostly an ixocutis or an ixotrichoderm, rarely a cutis or trichoderm; *clamp connections* present; development gymnocarpic to pseudoangiocarpic and stipitocarpic. Solitary to gregarious, terrestrial, always near trees or shrubs and apparently ectomycorrhizal principally with Pinaceae, Betulaceae and Fagaceae. Mostly in temperate zones of the Northern Hemisphere, but some taxa in similar climatic regions of Southern Hemisphere.

One species known for Tasmania.

24. *Hygrophorus involutus* G.Stev., *Kew Bull.* 16: 373 (1962). *Type*: New Zealand. Butterfly, 2.vi.1958, *G. Stevenson* (*Stevenson 1347*) (holotype K).

Pileus convex, white, yellow or apricot-yellow, viscid; trichoderm present; stipe concolorous with pileus.

Key to varieties of Hygrophorus involutus

1. Pileus, lamellae and stipe pure white .....**24a.** var. ***albus***
1. Pileus, lamellae and stipe shades of creamish yellow to apricot-yellow ....**24b.** var. ***involutus***

24a. *Hygrophorus involutus* G.Stev. var. ***albus*** A.M.Young & A.K.Mills, *var. nov.*
A *H. involutus* basidiomatibus albis differt.

Type: Tasmania. Arve Loop Rd nr Geeveston, *A.K.Mills* (*hb. Mills: 1541*) (holotype HO *508602*; isotype *hb.Mills*.)

Illustration: Fuhrer & Robinson (1992), p. 39 as *Camarophyllus niveus*.

Basidiomes wholly pure white but otherwise identical to those of var. *involutus*.

Habitat and distribution: gregarious amongst soil, humus or moss in cool temperate rainforest. The variety is reasonably common and often appears amongst normally coloured basidiomes.

Material: Myrtle Forest Track nr Collinsvale, 18.iii.1999, *G.Gates (hb. Mills 1628*; HO *508614*).

Remarks: The pure white basidiomes of *Hygrophorus involutus* var. *albus* differ from the normal light orange to yellowish basidiomes of *H. involutus* var. *involutus* only in that they are without any coloured pigments. Only two collections are here listed, however anecdotal evidence indicates that this white variety is widespread in suitable Tasmanian habitats.

24b. *Hygrophorus involutus* G.Stev. var. ***involutus***

Illustrations: Fuhrer & Robinson (1992), p. 45; Young & Wood (1997), p. 1020.

Habitat and distribution: gregarious amongst soil, humus or moss in cool temperate rainforest. This species is widespread and common in southern, central and north-western Tasmania.

Material: Arve Loop Rd nr Geeveston, 27.iv.1998, *A.M.Young* (*hb. Young 1958*; MEL *2087781*); Mt Field, 30.iv.1998, *A.M.Young* (*hb. Young 1972*; BRI); Little Florentine River, 13.v.1998, *A.M.Young* (*hb. Young 2040*; BRI); Growlingswallet nr Mt Field, 19.v.1999, *A.M.Young* (*hb. Young 2233*; MEL *2087770*); Arve Loop Rd nr Geeveston, 11.v.1998, *A.K.Mills* (*hb. Mills 1542*; HO *508603*); Arve Loop Rd nr Geeveston, 11.v.1998, *A.K.Mills* (*hb. Mills 1544*; HO *508604*); Sandspit Forest Reserve nr Wielangta, *A.K.Mills* (*hb. Mills 1588*; HO *508607*); Myrtle Forest Track nr Collinsvale, *G.Gates* (*hb. Mills 1627*; HO *508613*); Franklin R., 13.iv.1991, *E.Horak* (ZT *4317*; BRI).

Remarks: *Hygrophorus involutus* remains the sole representative of the genus for Australia. Collection *hb. Young 2040* was used to confirm previous remarks in Young (2000a) regarding the weakly divergent trama. Inspection has shown that the divergent structure is best observed if the section is taken from the larger lamellae of older pilei and at a point about one third of the distance of the pileus radius commencing at the margin. The divergent hyphae can be seen in the middle part of the lamellae.

Several Tasmanian collections exhibit basidiomes which have smaller mean spore sizes and Q's (5.5–7.5 × 3.5–4.5 µm, mean 6.5 × 4.2 µm, Q: 1.3–1.8, mean Q: 1.55) than those previously reported (Young & Wood 1997) from mainland material (6.0–7.5 (–9) × (3–)3.5–4.5(–5) µm, mean 7.2 × 3.9 µm, Q: 1.4–2.3, mean Q: 1.9). However, because there is so much overlap of dimensions and since all other aspects of the basidiomes are similar, the difference is considered to be normal species variation.

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Genetic evidence supports reclassification of *Agrostis billardierei* var. *filifolia* and *A. aemula* R.Br. var. *setifolia* as a single species, *A. punicea* (Poaceae)

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Abstract

The genetic relationship between *Agrostis billardierei* R.Br. var. *filifolia* Vickery and *A. aemula* R.Br. var. *setifolia* Vickery was investigated using random amplification of polymorphic DNA. Three random primers generated 49 products of which only one was present in all samples. *Agrostis aemula* var. *setifolia* was found to be more similar to *A. billardierei* var. *filifolia* than to *A. aemula* var. *aemula* supporting reclassification of *A. billardierei* var. *filifolia* and *A. aemula* var. *setifolia* as a single species, *A. punicea* A.J.Brown. & N.G.Walsh.

Introduction

Many populations of native grass species exist as isolated remnants because the once extensive grasslands on the western basalt plains in Victoria have declined to less than 1% of their previous extent (Scarlett *et al.* 1992). Interest in the preservation and utilisation of these species in Australia has highlighted some problems in the delineation of some taxa. Before management strategies for maintaining biodiversity can be implemented, there is a need to improve our understanding of the relationships between taxa and the level of diversity within recognised taxa.

A number of native species of *Agrostis* R.Br. are found in lowland Victoria: *A. adamsonii* Vickery, *A. aemula* R.Br., *A. avenacea* J.F.Gmel., *A. billardierei* R.Br., *A. robusta* A.J.Brown & N.G.Walsh, *A. punicea* A.J.Brown & N.G.Walsh, *A. rudis* Roem. & Schult. and *A. venusta* Trin. *Agrostis punicea* is a recently described species based on morphological examination of former varieties of *A. aemula* and *A. billardierei* (Brown & Walsh 2000). Doubts had existed about the legitimacy of *A. billardierei* var. *filifolia* Vickery and *A. aemula* var. *setifolia* Vickery because they are superficially very similar and their separation is based on small morphological differences. When describing the two taxa, Vickery (1941) noted that they showed a strong resemblance with the major difference being the hairy lemma of *A. aemula* var. *setifolia*. Brown and Walsh (2000) found that, besides the difference in lemma hairiness, *A. billardierei* var. *filifolia* and *A. aemula* var. *setifolia* were separated morphometrically, on average, only by the slightly shorter inflorescences and slightly longer lemmas, lemma-setae and awns of the latter taxon.

Brown and Walsh (2000) also found that *A. billardierei* var. *filifolia* and *A. aemula* var. *setifolia* had similar ecological preferences. While populations commonly contain either one of these taxa, populations have been observed where both occur, suggesting that they may interbreed. In contrast, the typical varieties of each species are generally distinct in form and growth habit (Walsh 1994).

The distribution of the taxa is somewhat different (Brown & Walsh 2000). *Agrostis. aemula* var. *setifolia* and *A. billardierei* var. *filifolia* is confined to moist, generally open, lowland environments of southern Victoria, south-east South Australia and Tasmania. *Agrostis aemula* var. *aemula* is fairly common from coastal to subalpine environments

across Victoria, New South Wales, South Australia and Tasmania with isolated occurances in Western Australia and Queensland. *Agrostis billardierei* var. *billardierei* is widely distributed around the coastlines of southern Australia with a few inland occurrences.

This study was undertaken to see whether there was genetic support for the reclassification by Brown and Walsh (2000) of *A. billardierei* var. *filifolia* and *A. aemula* var. *setifolia* as a single species, *A. punicea*. The similarity between these taxa is investigated using RAPDs which are dominant markers thought to sample randomly across the genome (Stammers *et al.* 1995). RAPDs have been found to be useful markers for taxonomic studies at the species level or for species complexes where the number of morphological characters can be insufficient between taxa and other DNA-based methods do not detect sufficient variation (Gonzalez & Ferrer 1993; Kazan *et al.* 1993; Stammers *et al.* 1995).

Agrostis avenacea was included for comparison in this study because it is considered a distinct though variable, common and widespread species, found in all regions of Victoria, in all states except the Northern Territory, and in Polynesia (Walsh 1994). Also, it frequently occurs near *A. aemula* and *A. billardierei*. It is distinguished from *A. aemula* by a number of features used collectively, rather than a sharp discontinuity in features, suggesting differences in the genetic basis of many genes which characterise the species. An earlier study on genetic diversity in native *Agrostis* species found that *A. avenacea* was less similar to *A. billardierei* var. *filifolia* than to *A. billardierei* var. *robusta* Vickery or *A. adamsonii* (James & Brown 2000).

Methods

PLANT MATERIAL

Wild populations of the study species were sampled from sites in western Victoria (Fig. 1). Seed collections for the populations, 'HD': Hadden (*Agrostis billardierei* var. *filifolia*), 'EM': East Mortlake (*A. aemula* var. *aemula*), 'WM': West Mortlake and 'SHR': Ballyrogan (*A. avenacea*) were made between December and February 1996/97. *Agrostis avenacea* was represented by both large weeping and small upright forms (WM and SHR respectively). Currently, these forms are not formally recognised. Seed was collected from populations 'SBL': South Bulart and 'LR': Lake Repose, Glenthompson (*A. billardierei* var. *filifolia*) and 'DIG': Dartmoor (*A. aemula* var. *setifolia*) between December and February 1998/99. Seed was obtained from up to 10 individuals in each population, with collections maintained as individual seed-lots. Approximately 20 seeds per individual were placed in tapering tubes in a pinebark-based medium, covered in a thin layer of mix with particle size < 2mm and left to germinate in a glasshouse for up to 10 weeks. A single seedling per individual seed-lot was chosen at random and potted on to provide fresh leaf material for DNA extractions.

DNA ISOLATION

Genomic DNA was extracted from fresh leaf material using acetylmethylammonium bromide (CTAB) method modified from Rogers and Bendich (1985). DNA quality was assessed visually after electrophoresis on a 1.0% agarose gel containing ethidium bromide. To further assess DNA quality, 0.5 µg DNA from a subsample of plants was digested separately with restriction enzymes *Eco*R1 and *Dra*1 in 25 µl volumes in the appropriate buffers at 37°C overnight (c. 17 h) and OD_{260}/OD_{280} ratio was measured.

DNA AMPLIFICATION

Forty synthetic decamer primers (kits A and B) from Operon Technologies, Inc. (Alameda, Calif., U.S.A.) were tested in PCR reactions according to the protocol of Williams *et al.* (1990). Three primers (OPB-8, OPB-12, OPB-20) giving discrete, reproducible amplification products were chosen for further analysis. Reactions were performed in a volume of 25 µl containing 5–10 ng DNA, 1 U Taq polymerase (Gibco-BRL),

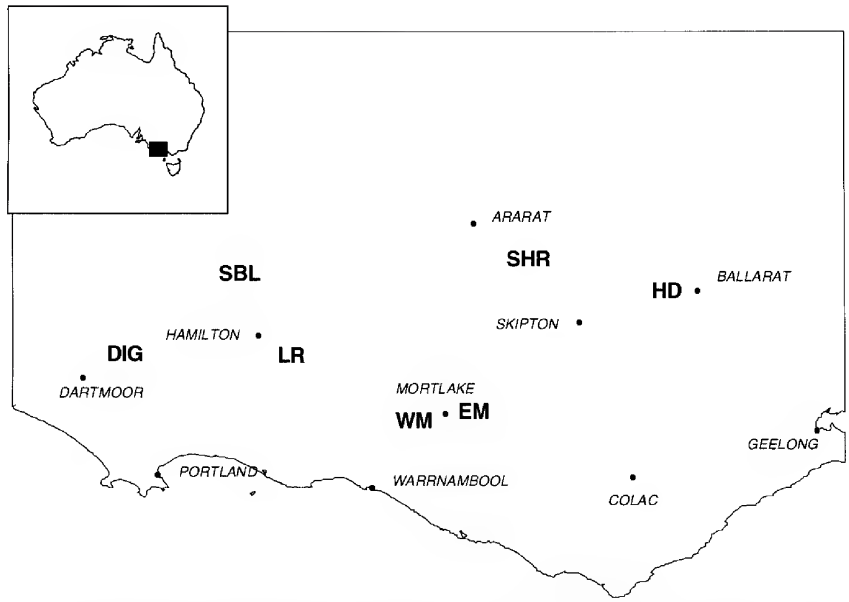

Figure 1. Distribution of *Agrostis* study sites in Victoria's Western District. *A. aemula* var. *aemula*: Wooriwyrite (EM); *A. aemula* var. *setifolia*: Digby (DIG); *A. avenacea*: Ballyrogan (SHR), Connewarren (WM); *A. billardierei* var. *filifolia*: Haddon (HD), Lake Repose (LR), South Bullart (SBL).

1 × Taq polymerase buffer (Gibco-BRL), 160 μM each dATP, dCTP, dGTP and dTTP, 1.5 mM $MgCl_2$ and 15ng primer, using a Corbett Research FTS 960 thermocycler. For the PCR, initial strand separation and amplification was initiated with one cycle of 94°C for 5 min, 35°C for 2 min, 72°C for 1 min, followed by 40 cycles of 94°C for 30 sec, 38°C for 30 sec (except for primer OPB-08 where annealing temperature was 44°C), 72°C for 30 sec. and a final extension step of 72°C for 3 min. Amplified products were resolved by electrophoresis on a 2% agarose gel containing ethidium bromide and run in TAE buffer at 80-100 v for 2 – 4 h. Images were visualised and photographed using a Sci Tech CCD video camera module and saved to computer using UVIdoc image capture software.

DATA ANALYSIS

RAPD products were scored as either present (1) or absent (0) for each individual, based on the assessment of a minimum of two independent PCR runs. Fragments migrating at the same size were considered to be homologous.

Genstat 5 version 4.1 (PC/Windows NT) (Lawes Agricultural Trust Rothamstead) was used for data analysis. Chi-squared tests were performed to determine which band frequencies were different between populations. Two similarity matrices were calculated using simple-matching coefficient (SMC) (Gordon 1981). The first compared individuals and was used as the basis for ordination by principle co-ordinate analysis (Gower 1966). The second compared populations. A dendrogram (average-link) was constructed based on the second, reduced similarity matrix, to compare populations. Diversity indices were calculated using the Shannon-information index (Russell *et al.* 1993) and presented as a single index, averaged over all loci, to provide an average per locus diversity (Chakraborty & Rao 1991). The amount of variation partitioned within and among populations was calculated from the diversity indices (King & Schaal 1989).

Results

Genetic variation was detected by RAPD PCR. RAPDs generated reliable and reproducible polymorphic patterns that enabled an assessment of the relationship between the former species: *A. aemula* (including both var. *aemula* and var. *setifolia*), *A. billardierei* (only var. *filifolia* represented) and *A. avenacea*.

Scorable RAPD fragments ranging from 0.38 to 2.5 kb in size were amplified by three decamer primers (OPB-08, -12 and -20). They generated a total of 49 products with an average of 16 products per primer. A fourth primer, whilst successfully amplifying DNA for most samples did not reliably amplify DNA from population 'EM' and so was eliminated from the analyses. The three primers revealed polymorphisms among the three species examined and only one amplification product was present in all individuals regardless of species. Polymorphic products per population varied from 0% (SBL) to 81.3% (SHR), and per species varied from 54.5% (*A. billardierei*) to 96.4% (*A. aemula*) (Table 1). The high level of polymorphism in *A. aemula* was due to the small number of RAPD fragments (3) that occurred in both the 'DIG' and 'EM' populations.

Table 1. Number of RAPD bands (% polymorphic), range in no. band differences between RAPD phenotypes* for each population and each species. *(populations only)

Species		Popn	Within populations		Within species
			No. RAPD bands (% polymorphic)	Range in no. band differences between RAPD phenotypes	No. RAPD bands (% polymorphic)
A. billardierei	var. *filifolia*	HD	14 (14.3)	1–2	
	var. *filifolia*	SBL	15 (0.0)	0–0	22 (54.5)
	var. *filifolia*	LR	20 (45.0)	1–9	
A. avenacea	upright form	SHR	17 (82.3)	1–11	24 (87.5)
	weeping form	WM	19 (63.2)	1–6	
A. aemula	var. *aemula*	EM	18 (66.7)	1–10	28 (96.4)
	var. *setifolia*	DIG	13 (15.4)	1–2	

Similarity between individuals ranged from 41.5–100%. Seventy-nine percent of RAPD PCR products varied significantly ($P = 0.01$) in frequency between populations. The average similarity between species was 56.2% for *A. billardierei* vs *A. avenacea*, 66.7% for *A. billardierei* vs *A. aemula* and 70.5% for *A. avenacea* vs *A. aemula*. The number of phenotypes present in populations is listed for each population (Table 2).

Similarity between populations ranged from 50.0-94.3% (Table 3). Of note, is the low similarity (62.0%) between the populations DIG and EM formerly considered to be different varieties of *A. aemula*. Likewise, similarity between EM (*A. aemula* var. *aemula*) and *A. billardierei* var. *filifolia* is only 51.8%. In contrast, the similarity between DIG (*A. aemula* var. *setifolia*) and *A. billardierei* var. *filifolia* is 81.7%.

The relationship between populations is depicted in Fig 2. The population of *A. aemula* var. *setifolia* (DIG) clearly clusters with the populations of *A. billardierei* var. *filifolia* (HD, SBL and LR). Importantly, DIG shows a greater affinity with HD (84.0%) rather than EM (62.0%) despite HD and DIG being separated by almost twice the geographic distance separating DIG and EM (Fig. 1).

Table 2. Sample numbers, population size and genetic diversity indices for each population.

Variety		Population (popn size)	No. samples	No RAPD phenotypes	H_O
A. billardierei	var. *filifolia*	HD (100-250)	9	4	0.0097
	var. *filifolia*	SBL (500-1000)	7	1	0.0000
	var. *filifolia*	LR (>2500)	7	6	0.0487
A. avenacea	upright form	SHR (100-250)	10	9	0.0808
	weeping form	WM (50-100)	10	9	0.0539
A. aemula	var. *aemula*	EM (100-250)	10	10	0.0648
	var. *setifolia*	DIG (>2500)	10	4	0.0068

Table 3. Reduced similarity matrix based on RAPD data comparing all populations. * = no. plants sampled.

Species	*A. billardierei*			*A. avenacea*		*A. aemula*	
Popn	HD[9]*	SBL[7]	LR[7]	SHR[10]	WM[10]	EM[10]	DIG[10]
HD	–						
SBL	85.6	–					
LR	84.8	94.3	–				
SHR	59.5	56.2	56.3	–			
WM	58.0	54.8	52.4	85.7	–		
EM	52.6	52.7	50.0	78.0	78.2	–	
DIG	84.0	81.2	79.8	62.5	63.1	62	-

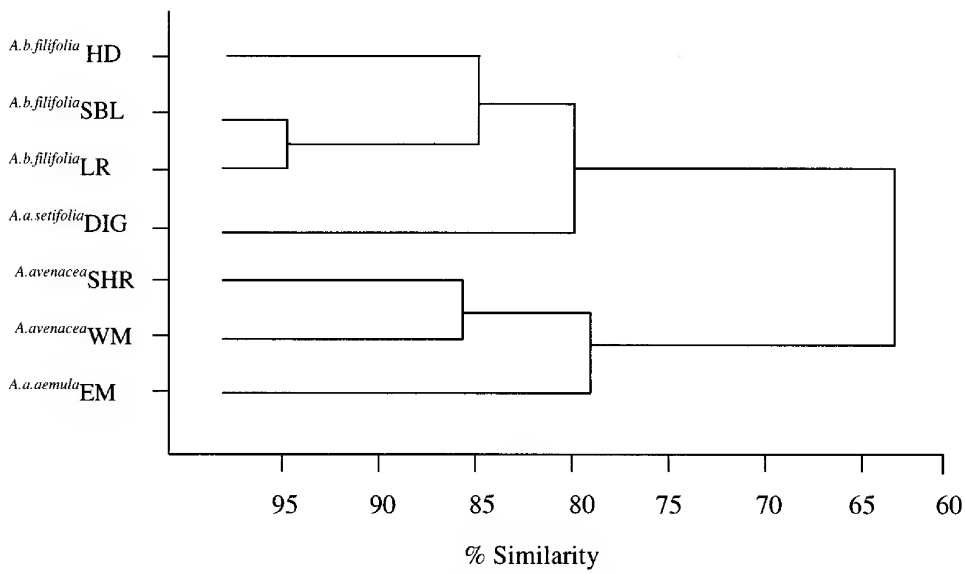

Figure 2. Cluster of populations based on reduced similarity matrix (simple matching coefficient).

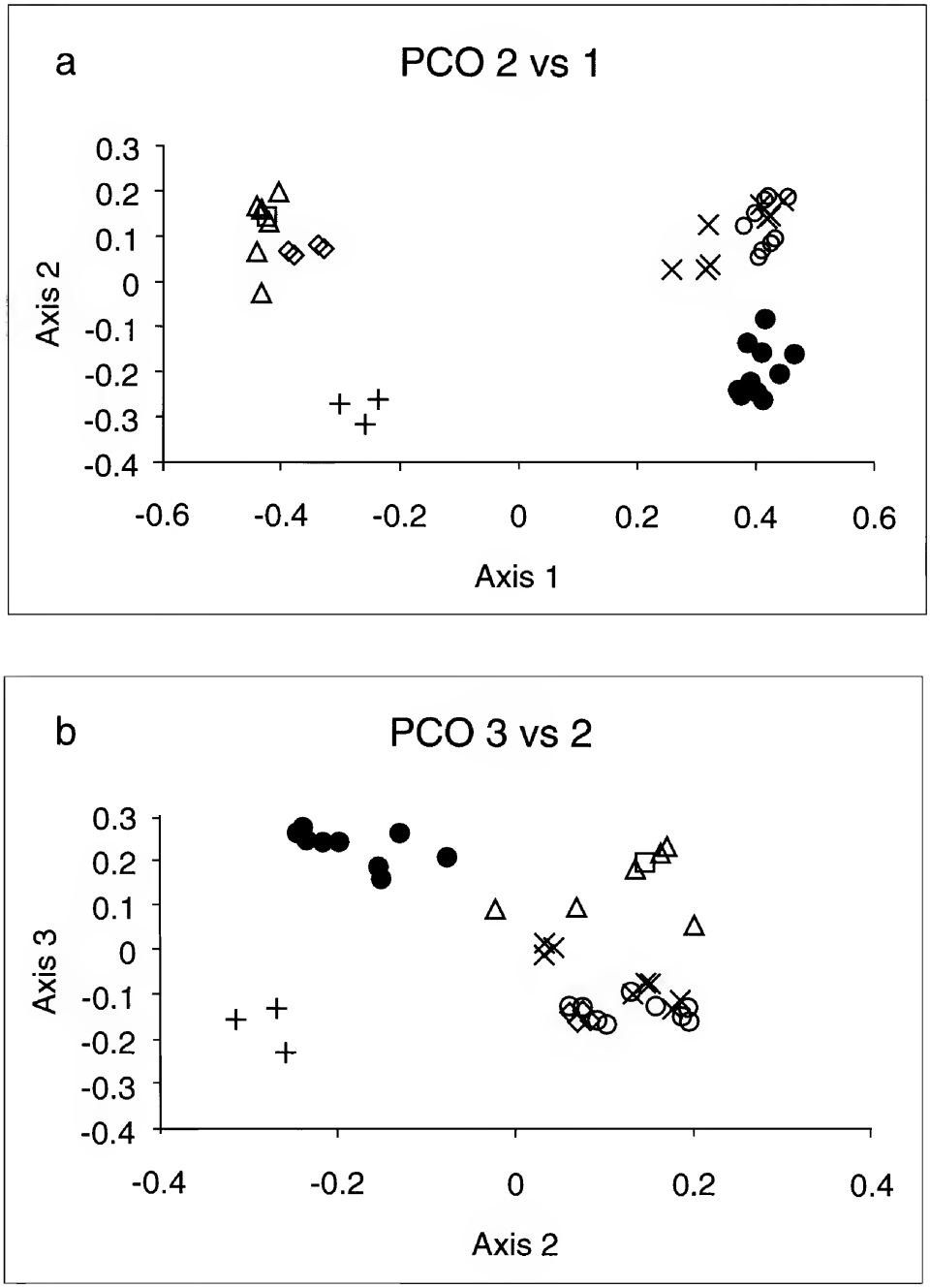

Figure 3. Principle co-ordinate analysis of RAPD phenotypes for all *Agrostis* individuals. Axes 1, 2 and 3 account for 73.8 % of variation. **a.** PCO vector 2 vs 1; **b.** PCO vector 3 vs 2. ◇HD, □SBL, △LR, ×SHR, ○WM, ●EM, +DIG. (Number of symbols does not equal number of individuals due to some individuals sharing phenotypes).

PRINCIPAL COORDINATE ANALYSIS OF INDIVIDUALS

The first two axes of the principal co-ordinate analysis accounted for 52.4% and 11.3% of total variation, respectively, with a further 9.9% accounted for by the third axis. Individuals within the populations of *A. billardierei* var. *filifolia* (HD, SBL and LR) group together and the populations form a discrete cluster (Fig 3a). Clustering for *A. avenacea* is similar, although some separation of the SHR population has occurred. However, for *A. aemula*, whilst the individuals within each variety cluster together (populations EM and DIG), the two varieties form two distinct and quite separate clusters in both principal co-ordinate plots (Figs 3a, b).

DIVERSITY INDICES

Estimates of individual population diversity (H_O) were derived from RAPD phenotypes (Table 2). The highest value of H_O=0.0808 was for population SHR of *A. avenacea*. No variation was detected in SBL and low levels were found in HD and DIG. While levels of diversity were variable depending on populations, they were not necessarily correlated with population size.

Average diversity for each species was calculated separately as $^{Abill}H_{SP}$=0.1526 for *A. billardierei*, $^{Aaven}H_{SP}$=0.2281 for *A. avenacea* and $^{Aaem}H_{SP}$=0.2722 for *A. aemula* (Table 4). The lower value for *A. billardierei* reflects the low population diversity values for populations HD and SBL. The comparatively high value for *A. aemula* results from the two populations sharing very few RAPD characters, whereas for *A. avenacea*, it results from high diversity within each population.

Average genus diversity (calculated for all populations combined) was $^{ALL}H_{GEN}$=0.2559. Average species diversity (calculated for all populations combined) was $^{ALL}H_{SP}$=0.2176.

H_{POP} provided measures of the average population diversity for each species, ranging from 0.0427 to 0.1540, and for the genus, 0.0378.

PARTITIONING OF VARIATION

Partitioning of variation within and among populations and species can provide an insight into their genetic structure. When variation is partitioned within and among populations (H_{POP}/H_{GEN}), using all seven populations in the analysis, only 14.8% of the observed variation is found within populations (Table 4). The proportion of diversity varies with species (Table 4). For *A. billardierei,* 28.0% of variation was present within populations whereas only 21.8% was present within populations of *A. aemula*. When indices are calculated for *A. billardierei* var. *filifolia* and *A. aemula* var. *setifolia* as a single species, the within-population variation decreases to 17.4%. For *A. avenacea*, 67.5% of variation was found within populations.

Table 4. Genetic diversity indices and partitioning of variation for species analysed separately and also combined.

Species	Diversity index	Partitioning of variation	
		Within popns	Among popns
Agrostis spp. combined	$^{ALL}H_{POP}$=0.0378 $^{ALL}H_{GEN}$=0.2559	14.8%	85.2%
A. billardierei	$^{Abill}H_{POP}$=0.0427 $^{Abill}H_{SP}$=0.1526	28.0% (17.4%)*	72.0% (82.6%)*
A. avenacea	$^{Aaven}H_{POP}$=0.1540 $^{Aaven}H_{SP}$=0.2281	67.5%	32.5%
A. aemula	$^{Aaem}H_{POP}$=0.0593 $^{Aaem}H_{SP}$=0.2722	21.8%	78.2%

*Partitioning of variation when population DIG is included with *A. billardierei*.

Discussion

Grass species have often been difficult to define because of minor differences in morphology, phenotypic plasticity in response to the environment, and frequently, a degree of intergradation between species. An advantage of using RAPDs is that they can be used to determine the affiliation of plants showing morphological characters with values common to more than one taxon. This approach has been used successfully for a number of grass species: *Agrostis adamsonii* (James & Brown 2000), *Hordeum* (Gonzalez & Ferrer 1993), *Lolium/Festuca* complex (Stammers *et al.* 1995; Pašakinskienė *et al.* 2000). The use of a molecular method in this study complements the set of characters used in the morphological analysis of Brown and Walsh (2000).

COMPARISON OF *AGROSTIS AEMULA* VAR. *SETIFOLIA* AND *A. BILLARDIEREI* VAR. *FILIFOLIA*

Agrostis aemula var. *setifolia* has been shown to be morphologically distinct from *A. aemula* var. *aemula* (Brown & Walsh 2000) and *A. billiardierei* var. *filifolia* has been shown to be both morphologically and genetically distinct from other varieties of *A. billiardierei* (Brown & Walsh 2000; James & Brown 2000; Ryan, unpublished data). This study shows a high degree of genetic similarity between *A. aemula* var. *setifolia* and *A. billardierei* var. *filifolia*.

Hierarchical clustering of populations (Fig. 2) shows three main clusters. The three populations of *A. billardierei* var. *filifolia* plus the population of *A. aemula* var. *setifolia* form a cluster that separates from *A. avenacea* and *A. aemula* var. *aemula* at a similarity of 62.0%.

In the principal co-ordinate analysis, *A. aemula* var. *setifolia* (DIG) individuals are clearly separated from populations of *A. billardierei* var. *filifolia*, although the most similar population is always HD. The clustering of the *A. billardierei* var. *filifolia* and *A. aemula* var. *setifolia* populations with 79.8% similarity is strong evidence for their reclassification as a single species or at least as individual species; both separate from *A. billardierei* and *A. aemula*. Cluster analysis using morphological characteristics found the same relative clustering pattern for *A. billardierei* var. *filifolia*, *A. aemula* var. *setifolia* and *A. aemula* var. *aemula* but at greater similarities (87% between the first two taxa and 79% between the first two and the third) (Brown & Walsh 2000).

The two populations of *A. avenacea* (WM and SHR) cluster at 85.7% similarity, and can be regarded as a single species, despite showing morphological variation and being separated by 70 km. *Agrostis avenacea* shows a similarity of 78.2% to *A. aemula* and indicates a closer similarity than would be normally accepted as delineating species. As there is no sharp morphological separation between *A. avenacea* and *A. aemula* (Walsh 1994), their taxonomic status requires further investigation, using a wider range of populations than is reported on here.

PARTITIONING OF VARIATION

The genetic structure of species is determined by the evolutionary and ecological history of individual species. The breeding systems of grasses influence the partitioning of variation, with selfing species having more genetic divergence (nearly half) among populations than mixed-mating and outcrossing species (Godt & Hamrick 1998). For outcrossing grass species, most diversity is found within populations. For example, in *Hordeum spontaneum*, which is known to have high levels of selfing, 43% of variation was found within populations (Dawson *et al.* 1993) whereas 72.9–80.5% within-population-variation was found within *Buchloe dactyloides*, a diploid out-crossing species (Huff *et al.* 1993).

Only 14.8% of observed variation in the combined *Agrostis* species genetic analysis is found within populations (Table 4). This is similar to the value (14.3%) found in another study of *Agrostis* species (James & Brown 2000) and is consistent with the low level of gene flow which is expected if the populations studied, comprise different species.

The proportion of variation partitioned within-populations for *A. billardierei* var. *filifolia* (28.0%) and *A. aemula* (21.8%) is much lower than the value of 73% found for other grass species (Godt & Hamrick 1998), the 78% reported for outcrossing species (Hamrick

et al. 1991) or the near 50% values reported for selfing species. The results for these *Agrostis* species could be due to self-fertility and the result of limited gene exchange among populations due to fragmentation of habitats, or some asexual reproduction. The low levels of variation in populations HD and DIG (Table 2) support the low levels commonly found in species with restricted ranges (Hamrick *et al.* 1991) but the complete absence of variation in SBL also indicates possible differences in reproductive strategies.

In *A. avenacea*, the variation within populations (67.5%) is similar to that found previously for *A. avenacea* (57.9%) using RAPDs (James & Brown 2000). Although the level of selfing in *A. avenacea* is not known, the variation within populations is lower but still comparable to an out-crossing species. *Agrostis avenacea* is a widespread, more common species than the others studied here and although some selfing may occur, its gene flow may not be as restricted.

POPULATION SIZE, GENETIC DIVERSITY AND BREEDING SYSTEM IMPLICATIONS

The mating system of plants plays an important role in determining the genetic structure and diversity of populations. Compared to other plants, grasses, in general, have higher levels of genetic diversity but there is more genetic differentiation among populations. For example, about 27% of total genetic variation is partitioned among grass populations compared with 22% for other plant species (Godt & Hamrick 1998).

Differences in the amount of variation in the populations of *A. billardierei* var. *filifolia* suggest that there may be variations in the breeding system within this taxon. It also suggests a flexible breeding system. It has been found that selfing species of grasses are genetically depauperate, relative to mixed mating and outcrossing species, both within populations and within species and in general, most are annuals whose population sizes often fluctuate (Godt & Hamrick 1998). It is possible that some of the *Agrostis* populations are ephemeral and sourced from larger, permanent populations where reproduction is mainly sexual, but apomixis occurs periodically. Population LR has a high level of diversity (H_O=0.0487) and could consist of plants which almost always reproduce sexually. On the other hand, populations HD (H_O=0.0097) and SBL (H_O=0.0) may have breeding systems with more emphasis on apomixis or selfing. SBL plants were grown from seed collected from different parents and the lack of variation in the sample suggests that seed may be apomictic in origin and that the population was founded by seed from a single parent plant. Similarly for DIG, there may be years where seed production is largely apomictic, leading to a cohort of genetically identical seedlings. Alternatively, the low diversity in DIG may be a result of repeated genetic bottlenecks if plant numbers fluctuate widely over time.

If apomictic populations of *Agrostis* species do occur, or if apomixis is a component of the breeding system, the genetic structure of populations may change rapidly. These results highlight the need for detailed breeding system studies on individual species or groups to understand their genetic structure.

Conclusions

Genetic variation detected with RAPD-PCR supports the conclusions resulting from a morphological study by Brown and Walsh (2000) placing *A. aemula* var. *setifolia* and *A. billardierei* var. *filifolia* in a new species, *A. punicea*. Other taxonomic issues still exist in the lowland *Agrostis* species in south-eastern Australia and a critical appraisal of additional closely related taxa, including assessment of reproductive structures, ploidy levels and breeding systems is still required to resolve them.

Acknowledgments

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Note added in proof: Since completion and submission of the study reported in this paper, the genus *Lachnagrostis* has been recognised to occur in Australia (Jacobs, *Telopea* **9**, 439-448, 2001; Jacobs, *Telopea* **9**, 837-838, 2002). The current names for taxa referred to in the text, with synonyms in brackets, are *L. aemula* (R.Br.) Trin. (syn. *Agrostis aemula*), *L. billardierei* (R.Br.) Trin. (syn. *A. billardierei*), *L. filiformis* (Forst.) Trin. (syn. *A. avenacea*), *L. scabra* (Beauv.) Nees. ex. Steudel (syn. *A. rudis*), *L. adamsonii* (Vickery) S.W.L.Jacobs (syn. *A. adamsonii*), *L. robusta* (Vickery) S.W.L.Jacobs (syn. *A. billardierei* var. *robusta*), *L. punicea* ssp. *punicea* (A.J.Brown & N.G.Walsh) S.W.L.Jacobs (syn. *A. punicea* var. *punicea*, *A. aemula* var. *setifolia*) and *L. punicea* ssp. *filifolia* (Vickery) S.W.L.Jacobs (syn. *A. punicea* var. *filifolia*, A. *billardierei* var. *filifolia*).

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Notes on *Conothamnus* Lindl. with the description of a new section, sect. *Gongylocephalus* Craven (Myrtaceae)

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Abstract

New morphological observations of *Conothamnus* Lindl. are reported and the new section, *Gongylocephalus* Craven, is described to accommodate *Trichobasis* Turcz. *nom. illeg.* which has been included in *Conothamnus* without taxonomic recognition until now.

Introduction

Conothamnus Lindl. was established by Lindley in 1839, based upon the single species *C. trinervis* Lindl. In 1852 Turczaninow described the genus *Trichobasis* Turcz. with *T. aurea* Turcz. its sole species. The name *T. aurea* is typified by the KW set of the collection *Drummond 5th coll. 147*. Turczaninow's generic name is illegitimate, being a later homonym of *Trichobasis* Léveillé, published in 1849. Bentham (1867) in effect transferred *Trichobasis aurea* to the previously monotypic *Conothamnus* when he described *C. divaricatus* Benth. the name of which is typified by the K set of the type collection of *T. aurea*. In 1904 Diels added a third species to *Conothamnus*, *C. neglectus* Diels. All three species are restricted to the southwest of Western Australia.

Conothamnus, including *Trichobasis*, has been treated as one of the several genera related to *Melaleuca* L., from which it has been distinguished by previous authors (e.g. Bentham 1867; Johnson & Briggs 1983; Rye 1987) by possession of a single ovule in each locule compared to several ovules per locule in *Melaleuca*. My observations of the three species of *Conothamnus*, *C. aureus*, *C. neglectus* and *C. trinervis*, were incongruent with those generally recorded in the literature. Primarily, the differences noted between the three species of *Conothamnus* and *Melaleuca* are:

C. trinervis, *C. aureus*, *C. neglectus*: Ovules 2 per locule, one on each side of an axile-basal and sometimes flange-like placenta, laterally or laterally-basally attached. Seeds 1 or 2 per locule, semi-obovoid, or narrowly or flattened obovoid, concave on the placental side, the testa coriaceous or thinly coriaceous, white or whitish-brown. Abortive/sterile ovules not developing into chaff.

Melaleuca: Ovules several to numerous, irregularly arranged on an axile-median to axile-basal peltate placenta, basally attached. Seeds few per locule, angular oblong-obovoid to subobovoid, the testa membranous, brown. Abortive/sterile ovules developing into chaff.

Within *Conothamnus* two groups may be distinguished as follows:

C. trinervis: Flowers in triads. Placenta well developed and distinctly flange-like. Seed semiobovoid, strongly concave on the placental side, the testa coriaceous, white.

C. aureus, *C. neglectus*: Flowers in dyads. Placenta not well developed. Seed narrowly or flattened obovoid, the testa thinly coriaceous, white or whitish-brown.

The above differences are indicative of two evolutionary lines within *Conothamnus* and taxonomic recognition at sectional level is warranted. Although the epithet *Trichobasis* is available for use at infra-generic rank, the section that includes the nomenclatural type of *Trichobasis*, *T. aureus*, is described as new with a new epithet, *Gongylocephalus*. Features of the placentation and seed of *Conothamnus* and the type species of *Melaleuca*, *M. leucadendra* (L.) L., are illustrated in Fig. 1.

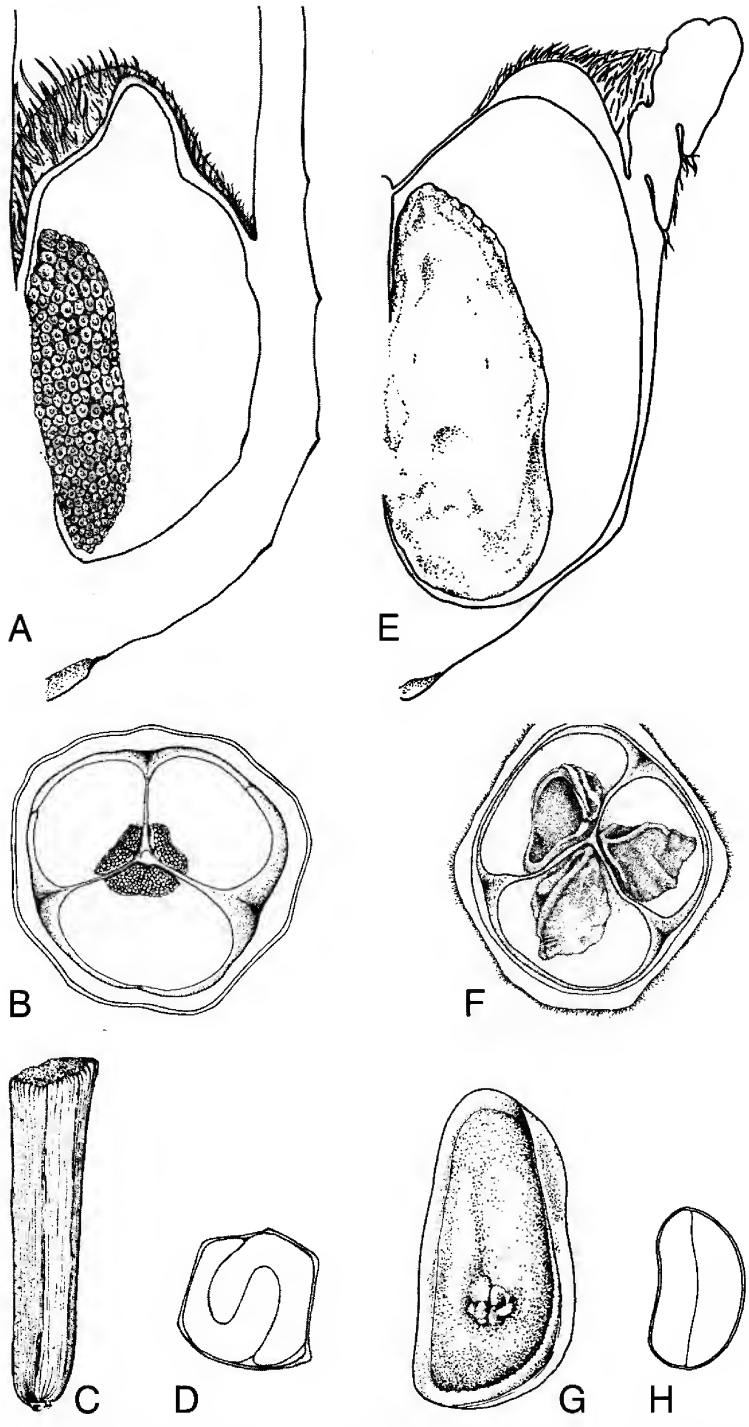

Figure 1. The ovary and seed of *Conothamus trinervis* (**E**, **F**, **G**, **H**) and *Melaleuca leucadendra* (**A**, **B**, **C**, **D**). **A**,**E**: longitudinal view through ovary locule; **B**, **F**: cross-sectional view through ovary locule (the flange-like placenta in F distorted as an artefact of drying). **C**, **G**: seed. **D**, **H**: cotyledon form. **A–D** from *Lazarides & Adams 322* (CANB); **E** from *Hoyle 211* (CANB); **F–H** from *Hnatiuk 760250* (PERTH).

Conothamnus Lindl., *Edwards's Bot. Reg. Appendix* vols 1–23: ix (1839). *Melaleuca* sect. *Conothamnus* (Lindley) Baill., *Hist. pl.* 6: 359 (1876). *Species typica*: *C. trinervis* Lindl.

Conothamnus sect. ***Conothamnus***

Shrubs. *Leaves* decussate or rarely ternate; venation parallel. *Inflorescences* capitate or spicate, pseudoterminal with the apex usually growing on after anthesis, several- to many-flowered. Flowers in triads, each triad subtended by a bract and with the constituent flowers all ebracteolate or variably bracteolate; not stipitate; perigynous. Hypanthium adnate to the ovary for the proximal 1/4 to 1/2 of the ovary. Sepals 5. Petals 5. Stamens indefinite; filaments fused into 5 antepetalous bundles; anthers dorsifixed, versatile, 2-celled, dehiscing by longitudinal slits. Ovary 3-locular; ovules 2 per locule, one each side of an axile-basal flange-like placenta, laterally attached. Stigma punctiform. *Fruit*, dry, not or scarcely woody, the locules dehiscing by valves. Seeds 1 or 2 per locule, semi-obovoid, concave on the placental side, the testa coriaceous, white. Embryo with the cotyledons about 1/2 the embryo length and flattened planoconvex.

C. trinervis Lindl., *Edwards's Bot. Reg. Appendix* vols 1–23: ix (1839). *Typus*: Western Australia, *Drummond s.n.* (holotypus CGE, *n.v*, photo in CANB).

Melaleuca cuspidata Turcz., *Bull. Soc. Imp. Naturalistes Moscou* 35: 327 (1862). *Typus*: Western Australia, *Drummond 7th coll. 77* (holotypus KW; isotypi MEL, NSW).

Conothamnus trinervis occurs in the Eneabba-Badgingarra district and in the Kalamunda area.

Conothamnus sect. ***Gongylocephalus*** Craven, *sect. nov.*

A sectione typica floribus duplicatis; petalis praesentibus vel carentibus; filamentis staminalibus liberis et staminis 5-aggregatis vel dispersis vel staminis coalitis in quinque fasciculis; stylis usque 5 mm longis; et placenta non distincte pteroidea differt.

Species typica: *Conothamnus aureus* (Turcz.) Domin

Trichobasis Turcz., *Bull. Cl. Phys.-Math. Acad. Imp. Sci. Saint-Petersbourg* 10: 337 (1852), *nom. illeg.*; non Léveillé (1849). *Typus*: *T. aureus* Turcz.

Shrubs. *Leaves* decussate to subdecussate; venation parallel-pinnate. *Inflorescences* capitate or spicate, pseudoterminal and sometimes also in distal leaf axils, with the apex usually growing on after anthesis, several- to many-flowered. Flowers in dyads, each dyad subtended by a bract and with the constituent flowers ebracteolate; stipitate or not; perigynous. Hypanthium adnate to the ovary for the proximal 1/4 to 2/3 of the length of the ovary. Sepals 5 (rarely 6). Petals 5 (rarely 6) or absent. Stamens indefinite; filaments free and the stamens grouped in antepetalous clusters (sometimes dispersed around the hypanthium apex) or fused in 5 antepetalous bundles; anthers dorsifixed, versatile, 2-celled, dehiscing by longitudinal slits. Ovary 3-locular; ovules 2 per locule, one on each side of a small axile-basal non-peltate placenta, laterally-basally attached. Stigma punctiform. *Fruit* dry, not or scarcely woody, the locules dehiscing by valves. Seeds usually 1 per locule, narrowly or flattened obovoid, the testa thinly coriaceous, white or whitish-brown. Embryo with the cotyledons about 1/2 the embryo length and flattened planoconvex.

The new sectional epithet is derived from the Greek *gongylos* (ball, sphere) and *kephale* (head) in reference to the shape of the inflorescence.

C. aureus (Turcz.) Domin, *Mem. Soc. Roy. Sci. Boheme. Prague* 1921–2 2: 91 (1923). *Trichobasis aurea* Turcz., *Bull. Cl. Phys.-Math. Acad. Imp. Sci. Saint-Petersbourg* 10:

337 (1852). *Typus*: Western Australia, *Drummond 5th coll. 147* (holotypus KW, *n.v.*, photo in PERTH *n.v.*; isotypi G, K *n.v.*, MEL, NY *n.v.*).

Conothamnus divaricatus Benth., *Fl. Austral.* 3: 164 (1867). *Typus*: Western Australia, *Drummond 5th coll. 147* (holotypus K, *n.v.*; isotypi G, KW *n.v.* photo in PERTH *n.v.*, MEL, NY *n.v.*).

This species occurs from the Stirling Range east to Israelite Bay.

C. neglectus Diels in Diels & Pritzel, *Bot. Jahrb. Syst.* 35: 430 (1904). Syntypi: Western Australia: Mt Melville near King George Sound, *Mueller s.n.* (B†, ?MEL *n.v.*); near Cranbrook, *Diels 4438* (B†; PERTH fragm); about 15 km N of Albany, *Diels 6034* (B†).

C. neglectus occurs from Walpole east to Albany and as far north as Borden.

The name *C. neglectus* is not being lectotypified here as a thorough search for other syntypes or isosyntypes has not been made. More ample materials of *Diels 4438* and/or *6034* than the fragment of *Diels 4438* in PERTH may exist. Material from Mount Melville, ascribed to Mueller by Diels in the protologue, has not been seen from MEL but, in view of the wide distribution of specimens from MEL to European and north American herbaria, such material may be extant.

Key to the species of *Conothamnus*

1. Flowers in triads; style 12-15 mm long (petals present) ..... *C. trinervis*
1. Flowers in dyads; style 2-5 mm long
   2. Petals absent; style 3.4-5 mm long ..... *C. aureus*
   2. Petals present; style 2-2.5 mm long ..... *C. neglectus*

Acknowledgments

The directors and curators of the following herbaria are thanked for the opportunity to study collections in their care: AD, BRI, CANB, KW, MEL, NSW, PERTH. Julie Matarczyk is thanked for her assistance with bibliographic work and data collection. The illustration was prepared by Rob Warren. Preparation of this paper in part was supported by the Australian Biological Resources Study.

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Muelleria 16: 43–45 (2002)

Calotis cuneata var. *pubescens* (Asteraceae), change in rank and notes on its distribution and ecology

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Abstract

Calotis cuneata (F.Muell. ex Benth.) G.L.Davis var. *pubescens* (F.Muell. ex Benth.) G.L.Davis is elevated to species rank as *Calotis pubescens* (F.Muell. ex Benth.) N.G.Walsh & K.L.McDougall and its ecology and conservation status are discussed.

Background

Until recently *Calotis cuneata* (F.Muell. ex Benth.) G.L.Davis var. *pubescens* (F.Muell. ex Benth.) G.L.Davis was known from only four collections: F. Mueller's 1854 type from 'grassy mountains on the Mitta Mitta', Victoria (MEL), two 1956 collections by M. Mueller from Nungar Plain and Snowy Plain, Kosciuszko National Park, New South Wales (CANB), and a 1967 collection by W. Bryant from Nungar Plain (NSW). The taxon has not been recollected in Victoria and may now be extinct in that state although Ross (2000) regarded it as 'poorly known'. Many likely areas of occurrence in F. Mueller's rather vague collection area are now largely converted to pasture, although the possibility exists that it still occurs on grassy plains of the Alpine National Park, Bogong Unit. Surveys by one of us (KMcD) in February 2001 on Nungar Plain, recorded *C. cuneata* var. *pubescens* at four sites, and further surveys by both of us in December 2001 located the taxon at 19 individual sites on the same plain. Surveys of similar subalpine grassy plains in the area (e.g. Long Plain, Happy Jacks Plain, Boggy Plain, Gulf Plain) have not resulted in further discoveries of the taxon. A survey in February 2001 of Snowy Plain in Kosciuszko National Park, where *C. cuneata* var. *pubescens* had been collected by M. Mueller in 1956, failed to locate the taxon (R. Rehwinkel, NPWS, pers. comm.).

The recent gatherings of the taxon have allowed more detailed comparisons with the typical variety of *C. cuneata*. Following these comparisons we are convinced that, despite superficial similarities in the mature cypselas, *C. cuneata* var. *pubescens* is rather distantly related to *C. cuneata* sens. str. and is at least as closely related to *C. scabiosifolia* Sond. & F.Muell. (within which Bentham (1867) originally included both varieties of *C. cuneata*).

In erecting *Calotis cuneata* Davis (1952) distinguished it from *C. scabiosifolia* (in which she retained two of Bentham's (1867) original six varieties) by the presence of a second series of fine plumose awns within the ring of peripheral awns on the apex of the cypsela. The two varieties of *C. cuneata* were distinguished by foliar characters, indumentum, and by the presence of a patch of appressed hairs on the central part of the body of the cypsela of var. *pubescens*. Although significant within *C. cuneata*, this last feature also occurs on *C. scabiosifolia* var. *integrifolia* F.Muell. ex Benth. The main cypsela awns of *C. cuneata* var. *pubescens* are unique amongst all four taxa within *C. scabiosifolia* and *C. cuneata* in being non-scabrous.

More detailed comparison of the cypselas of the two varieties of *C. cuneata* shows some significant discriminating features not noted by Davis or in the account of the genus by Everett (1992). The central awns of *C. cuneata* var. *cuneata* are united into a solid column in their basal half. This column encircles the base of the corolla. The central awns

of *C. cuneata* var. *pubescens* are much finer and free to their bases. The margins of the cypselas of var. *cuneata* are narrow and acute while those of var. *pubescens* are broadly thickened and rounded rather like those of *C. scabiosifolia*.

The general indumentum of *C. cuneata* var. *pubescens* differs from the typical variety and from both varieties of *C. scabiosifolia*. The last three taxa have strigose, septate hairs of varying density and coarseness, but all are evenly tapered from base to apex. *Calotis cuneata* var. *pubescens* has hairs with a coarse, erect septate base that is rather abruptly attenuated into a distinctly longer and finer apical part. This apical filament is often lost from the hairs of older and/or exposed parts of the leaves and stems leaving the persistent basal stub which results in a coarse hispid indumentum on these parts.

The distribution of *C. cuneata* as it is currently circumscribed gives a pattern that cannot be reconciled with a notion of relatively recent evolution of two entities from a common ancestor (as might be inferred from their varietal status). The typical variety is widely distributed from inland northern New South Wales to central Queensland while var. *pubescens* is highly localised in the subalps of north-eastern Victoria and southern New South Wales.

For the reasons outlined above we here elevate *C. cuneata* var. *pubescens* to specific rank.

Taxonomy

Calotis pubescens (F.Muell. ex Benth.) N.G.Walsh & K.L.McDougall, *stat. nov.*
Calotis scabiosifolia Sond. & F.Muell. var. *pubescens* F.Muell. ex Benth., *Fl. Austral.* 3: 503 (1867). *Calotis cuneata* (F.Muell. ex Benth.) G.L.Davis var *pubescens* (F.Muell. ex Benth.) G.L.Davis, *Proc. Linn. Soc. New South Wales* 77: 178 (1952). *Lectotype*: 'Grassy mountains on the Mitta Mitta', *F. Mueller s.n.*, 1854 (MEL) *fide* G.L. Davis *loc. cit.*

Ecology

At Nungar Plain, *C. pubescens* occurs in a herbfield community (in which it may be dominant) on gentle slopes between *Eucalyptus pauciflora* woodland and the valley floor which is vegetated by a mosaic of *Poa*-dominated tussock grasslands, open heaths dominated by *Hovea montana* and Cyperaceae-rich wetland communities. Soils are of the alpine humus type developed on a parent material of Silurian siltstone and shale of the Tantangara Formation. The altitude range is small, between c. 1340 and 1380 m a.s.l.

Colonies of *C. pubescens* may comprise a single genet developed by rhizomatous growth and can be up to 10m in diameter. Typically associated species include *Bulbine glauca, Coprosma nivalis*, *Leptorhynchos elongatus*, *Oreomyrrhis argentea, Plantago euryphylla, Poa petrophila, Poa hookeri* and *Wahlenbergia densifolia. Calotis glandulosa* F.Muell. is also relatively abundant on the plain. A comprehensive checklist of the flora of Nungar Plain will be published elsewhere (McDougall & Walsh in prep.).

Conservation Status

Based on Briggs and Leigh (1996), an appropriate conservation code for *Calotis pubescens* is 3ECi. The species is threatened by feral pigs, which have excavated large areas of vegetation on Nungar Plain, especially the herbland community containing *C. pubescens*.

Acknowledgements

We are grateful to the two anonymous referees for useful comments on drafts of this paper.

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Muelleria 16: 47–53 (2002)

Successful DNA amplification from *Acacia* (Leguminosae) and other refractory Australian plants and fungi using a nested/semi-nested PCR protocol

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Abstract

Despite the promise of the polymerase chain reaction (PCR) as a method for rapid generation of informative phylogenetic characters, it has proven difficult or impossible to amplify the commonly used internal transcribed spacer (ITS) 1+2 regions using standard approaches for several groups of Australian plants and fungi. This paper describes a two step nested or semi-nested PCR protocol that was utilised to provide the first consistent amplification of the ITS region from *Acacia* Mill. (Mimosoideae: Leguminosae). Subsequently these methods have proven to provide reliable amplification of ITS from previously intractable Australian members of the Rutaceae and Cortinariaceae. In addition to use with fresh material, this method has also proven effective with herbarium material up to 26 years old. It is hoped these methods may facilitate use of the ITS region from a broader range of plants and fungi, including herbarium material.

Introduction

In recent years the use of PCR for obtaining DNA sequences for systematics has expanded greatly. Of the DNA regions sequenced, the internal transcribed spacer (ITS) region of 18S-26S nuclear ribosomal DNA has proven to be particularly useful for plant (Baldwin *et al.* 1995) and fungal (Edel 1998) systematics. The relatively small size of ITS and its high copy number facilitates its amplification and sequencing (Baldwin *et al.* 1995), and rapid concerted evolution of the nuclear ribosomal DNA multigene family acts to promote intragenomic uniformity of repeats (Arnheim *et al.* 1980; Appels & Dvorak 1982; Arnheim 1983; Hillis *et al.* 1991), decreasing the likelihood of paralogy confounding phylogenetic reconstructions based on ITS sequences. Ease of alignment, and sufficient sequence variation between closely related species, has also contributed to the use of ITS for systematics. The popularity of ITS has resulted in more than 45,000 records being submitted to the GenBank DNA sequence database, which makes comparison of sequences with other taxa, worldwide, extremely easy.

The many advantages of the ITS region has seen it used for examining relationships between several Australian plant groups, e.g. Leguminosae: Papilionoideae (Crisp *et al.* 2000), *Hovea* R.Br. (Leguminosae, Thompson *et al.* 2001), *Beaufortia* R.Br. suballiance (Myrtaceae, Ladiges *et al.* 1999; Brown *et al.* 2001) and *Eucalyptus* L'Her. (Myrtaceae, Steane *et al.* 1999; Udovicic & Ladiges, 2000). Likewise, when it was necessary to sequence a region of nuclear DNA for acacias, ITS was deemed to be the ideal candidate. Initially, amplification of DNA from *Acacia* Mill. was attempted using primers that had proven successful for another legume genus, *Lupinus* L. (ITS18, ITS26, S3, S4, S5 and S6; Käss & Wink 1997). PCR reactions with these primers, in a variety of combinations, and using standard protocols either amplified nothing or multiple fragments. When single bands (from samples that had multiple fragments amplified) were isolated from agarose gel and sequenced, the sequences invariably matched fungal DNA sequences in GenBank. Consequently it was necessary to use primers more specific for higher plants in an attempt to avoid amplifying these fungal sequences by PCR.

Angiosperm-specific primers, 17SE and 26SE (Sun *et al.* 1994), were tried. These

primers also resulted in multiple PCR fragments and preferential amplification of unwanted fungal DNA or chloroplast ribosomal DNA. If higher annealing temperatures (above 62°C) were used, no DNA was amplified. When the 17SE and 26SE primer sequences were compared to the partial 18S and 26S sequences available for *Acacia* in GenBank - from *A. fimbriata* A.Cunn. ex G.Don (Martin & Dowd 1993) - it was found that there were four bp differences between the 18S sequence and the 17SE primer at the 5' end.

J. Miller (CSIRO Canberra) cloned the ITS region for several *Acacia* taxa and designed a new forward primer in the 18S (AcF). This primer was found to amplify the ITS region in combination with 26SE. High stringency PCR conditions (annealing temperatures above 60°C and hotstart PCR) were required to produce a single amplicon. Difficulties were experienced in obtaining a high yield of PCR product, especially when higher annealing temperatures were used. To overcome this problem a two step, nested or semi-nested PCR strategy was utilised, which incorporated a PCR additive (Q-solution, QIAGEN) to change the melting properties of the double stranded template DNA. The nested PCR approach was originally developed for detection of hepatitis C viral sequences in blood samples (Garson *et al.* 1990), and because of its sensitivity for amplification of low-copy number templates it is frequently used for characterisation and identification of micro-organisms. Here, the ultra-sensitivity of nested PCR is used to assist in amplification of the ITS region of Australian acacias, Rutaceae and fungi.

Materials and Methods

Material of *Acacia*, Rutaceae (*Phebalium* Vent. and allied genera) and fungi (Cortinariaceae: *Cortinarius* (Pers.) Gray and *Dermocybe* (Fr.:Fr.) Wünsche) was sampled from the field, living collections or herbarium collections. Fresh material was stored at 4°C until isolation of DNA was performed within a week of collection. Alternatively, material collected in the field was dried rapidly in silica gel and stored until convenient to isolate (Chase & Hillis 1991). Where possible herbarium material was chosen from collections under five years of age, although some collections sampled were up to 26 years old.

Genomic DNA was isolated using a CTAB protocol (Udovicic *et al.* 1995), followed by purification with Qiagen Tip 20 columns or by using Prep-a-Gene (Bio Rad). Herbarium samples were preferentially isolated using commercial kits, QIAGEN DNeasy mini kits or NucleoSpin kits (Macherey-Nagel, Düren, Germany), because of the small quantity of starting material needed and the consistent quality of resulting DNA.

The polymerase chain reaction (Mullis & Faloona 1987; Saiki *et al.* 1988) was used to amplify the ITS 1 + 2 region using a two step process (Fig. 1). Total volume of all DNA amplifications was 50 µl. First round reactions contained 0.2 mM dNTPs, 3 mM $MgCl_2$, 10 pmol each primer (Table 1), 1.25 units HotStar Taq DNA polymerase (Qiagen), 30–100 ng of template DNA and 10 µl Q-solution (Qiagen).

Thermal cycling was performed on an Eppendorf Mastercycler gradient thermal cycler with one hold at 95°C for 15 min preceding 30 cycles of 94°C for 30 s, 64°C for 30 s, 72°C for 20 s, and followed by one hold at 72°C for 5 min.

In second round reactions a 0.5–1 µl aliquot of PCR product from the first round reaction was used as the template. Alternatively, template DNA was obtained from agarose gels of first round reactions by "stabbing" the target band with a 10 µl pipette tip, followed by agitation in 20 µl of ultrapure water, 10 µl of which was used in the second round PCR. Apart from primers for nested or semi-nested PCR (Table 1), reaction components and conditions were unchanged from the first round. Products of amplifications were visualised by agarose gel electrophoresis and stained with ethidium bromide.

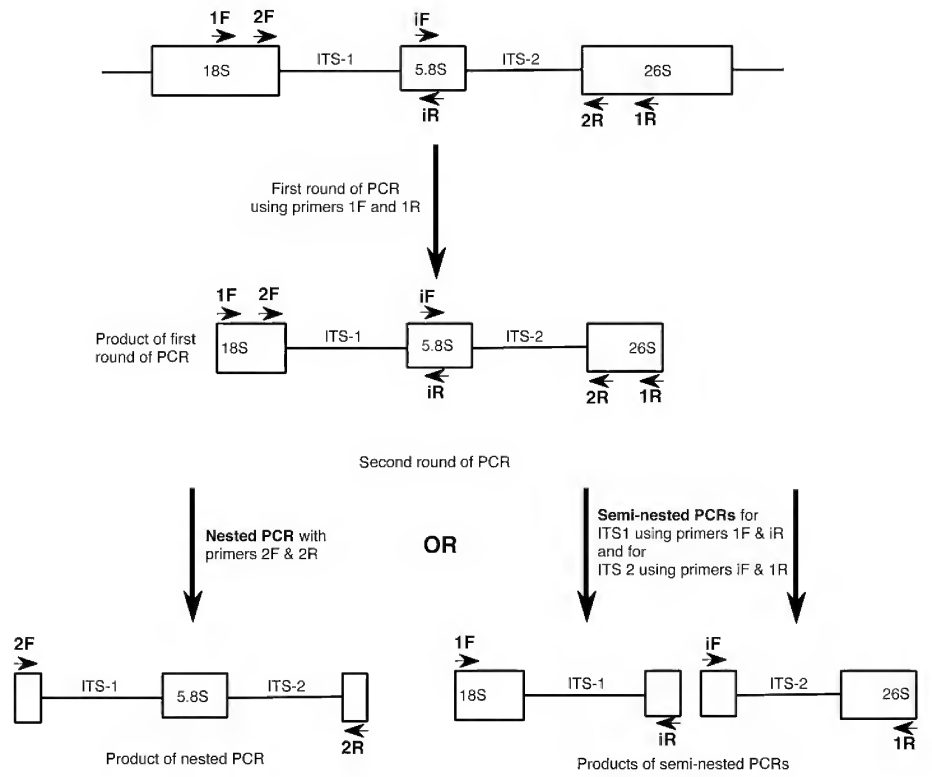

Figure 1. Diagram of nested and semi-nested PCRs, including location of primers. 1F and 1R denote primers used for the first round of PCR, 2F and 2R are primers used for nested PCR, and iF and iR are internal primers used for semi-nested PCR. See Table 1 for primers used for different taxa. Note that DNA regions and relative positions of primers are not to scale.

Table 1. PCR primers used for different taxa.

Taxa	1st Round Primers		2nd Round Primers		Internal Primers	
	1F	**1R**	**2F**	**2R**	**iF**	**iR**
Acacia	AcF[1]	26SE[2]	S3[3]	S4[3]	S6[3]	S5[3]
Rutaceae	ITS18[3]	ITS26[3]	S3[3]	S4[3]	S6[3]	S5[3]
Fungi	ITS1[4]	ITS4-B[5]	ITS1[4]	ITS4[4]	ITS3[4]	ITS2[4]

Notes: Origins of primers as denoted by superscript numerals: 1= J. Miller, CSIRO, Canberra; 2= Sun *et al.* (1994); 3= Käss and Wink (1997); 4= White *et al.* (1990); 5= Gardes and Bruns (1993).

Results and Discussion

For *Acacia*, the first round of amplification resulted in a DNA fragment of approximately 750–760 bp. This product was generally only present in low quantities and was difficult or impossible to visualise. The second round of nested PCR usually amplified a single fragment of DNA approximately 650 bp long (Fig. 2). If the second round of amplification resulted in a low yield of product (Fig. 2, lane 2), multiple fragments (Fig. 2, lane

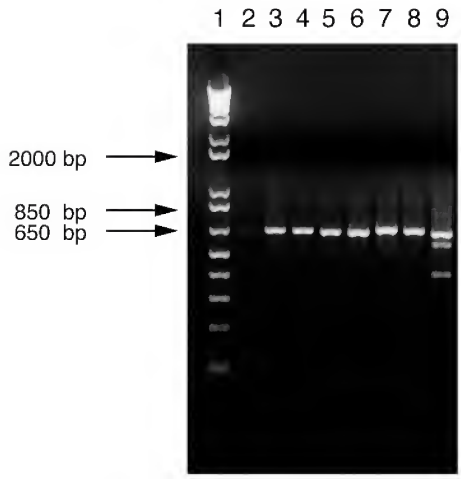

Figure 2. Second round of nested PCR amplification of *Acacia* DNA using primers S3 and S4. Aliquots of 5 µl taken from 50 µl PCR reactions, electrophoresed in a 1.5% agarose gel, stained with ethidium bromide and visualised with UV light.
1: 1kb Plus molecular weight ladder; 2: *Acacia verticillata* (L'Her.) Willd., note low yield of product; 3: *A. elata* Benth.; 4: *A. latisepala* Pedley; 5: *A. translucens* Cunn. ex Hook.; 6: *A. tumida* F.Muell. ex Benth.; 7: *A. ampliceps* Maslin; 8: *A. colei* Maslin & Thompson; 9: *A. platycarpa* F.Muell., note the multiple amplification products.

9), or was unsuccessful, semi-nested PCR was then used. Semi-nested PCR amplified fragments of approximately 400 bp for ITS 1 and 450 bp for ITS 2 (Fig. 3). Subsequent use of this protocol for Rutaceae and fungal DNA yielded comparable results to *Acacia*.

Primer selection was found to be of particular importance for amplification of ITS. Use of non-specific primers resulted in little or no amplification of the target template or preferential amplification of non-target templates. However, problems with amplification were not always due to non-specificity of primers; for example, in this study a new forward primer was synthesised to target the 3' end of the 18S rDNA gene of *Acacia.* Although being a perfect match to a published sequence for *A. fimbriata*, the *Acacia*-specific primer failed to amplify the ITS region. Such problems may be due to secondary structure in the target area of the primer. Alternatively, the species of *Acacia* trialed may have had mismatches at the priming site, although, this is unlikely given the conservative nature of the 18S rDNA sequence. In the case of Rutaceae, primers used for legumes (Käss & Wink 1997) worked satisfactorily for nested/semi-nested PCR. For fungi, primers ITS1 and ITS4-B were used for the first round of amplification. These were determined to give the best amplification of DNA from *Dermocybe* and *Cortinarius* after trialing the commonly used universal primers, ITS1 and ITS4 (White *et al.* 1990) and fungal (ITS1-F) and basidiomycete (ITS4-B) specific primers (Gardes & Bruns 1993) in all combinations.

The advantage of the nested PCR approach has been the ability to use a high annealing temperature, allowing high stringency PCR for avoidance of non-target amplification. Subsequent sequencing revealed a high G + C content (>70%) in the ITS region of *Acacia*, compared to a G + C content of 50–60 % in the fungal contaminants. This could explain the preferential amplification of contaminants at standard annealing temperatures, because at these temperatures unmelted G + C regions can prevent primer binding (Borman *et al.* 2000). Use of high annealing temperatures reduced the incidence of non-

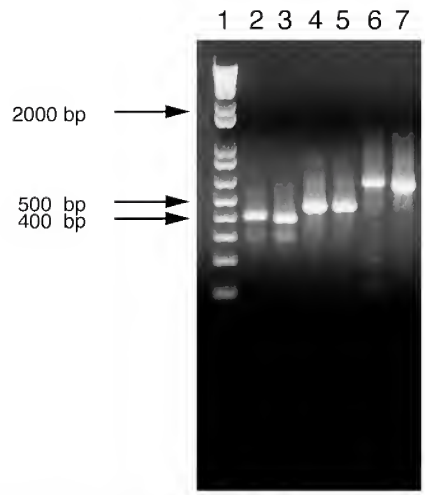

Figure 3. A comparison of PCR products resulting from semi-nested and nested amplification of *Acacia* DNA. Aliquots of 5 µl taken from 50 µl PCR reactions, electrophoresed in a 1.5% agarose gel, stained with ethidium bromide and visualised with UV light. Lane1: 1kb Plus molecular weight ladder; Lanes 2 and 3 show semi-nested PCR of ITS 1 for *A. mearnsii* De Wild. and *A. paradoxa* DC., respectively; Lanes 4 and 5 show semi-nested PCR of ITS 2 for *A. mearnsii* and *A. paradoxa*, respectively; Lanes 6 and 7 show nested PCR of ITS 1+2 for *A. mearnsii* and *A. paradoxa*, respectively.

specific amplification, and while resulting in a low yield in the first round of amplification because of the unstable nature of the primer and template hybrid (Varadaraj & Skinner 1994), the second round of amplification served to greatly increase the yield of desired product. Despite the high annealing temperature used for PCR, for *Acacia* a small proportion (approximately 10%) of second round amplifications resulted in non-target templates still being amplified (Fig. 2, lane 9). In these cases it was possible to amplify the *Acacia* ITS region using semi-nested PCR in the second round of amplification.

For *Acacia*, as described in materials in methods, aliquots of the PCR product from the first round of amplification served as the template for the second round of amplification, and this method was also used for fungal (*Dermocybe* and *Cortinarius*) DNA. Occasionally in Rutaceae this approach resulted in a background of smeared DNA, in addition to the expected product, when visualised on an agarose gel. This problem may be caused by nonspecific amplification in the original PCR (Hengen 1995), but this was unlikely in this case due to the high stringency conditions used. Another common reason for the occurrence of DNA smears is the use of far too much DNA template for the second round of amplification, but as found by Hengen (1995) dilution of the primary PCR product up to 10 000-fold may not resolve the smear into a discrete band after the second round of amplification. Alternatively, a greatly reduced amount of template for the second round PCR can be obtained by band-stab PCR, in which a needle or toothpick is used to "stab" the appropriate DNA band on a gel, and subsequently the needle or toothpick is dipped into the second round PCR reaction mix (Bjourson & Cooper 1992; Kadokami & Lewis 1994). This method achieves the aim of a greatly reduced amount of template for the second round PCR and isolates the desired fragment from the smeared DNA background. It is also quicker than cutting the desired band out of the gel and isolating it. Instead of toothpicks or needles, pipette tips were used to stab the band, and then, rather than mixing this directly into the PCR reaction, it was agitated in a separate tube con-

taining 20 µl of water. This allowed storage of this template DNA in a freezer for later use, and enabled multiple second round reactions to be carried out from the one sample.

A useful feature of nested PCR is the ability to amplify the ITS region from low concentrations of genomic DNA. As found in this study, this is particularly relevant when using DNA isolated from small amounts of material, such as herbarium specimens, which in addition, typically yield small quantities of degraded genomic DNA (Jansen *et al.* 1999). Previously, nested PCR has been used with some success for amplification of the *rbc*L region from DNA isolated from herbarium material (Savolainen *et al.* 1995). The use of herbarium material allows ready access to many taxa, including rare or even extinct species (Jansen *et al.* 1999). These specimens are already vouchered and have often been identified by specialists, thus increasing the reliability and repeatability of molecular systematic research. The use of herbarium material is time and cost effective, reducing the need to re-collect specimens (Wood *et al.* 1999). The nested PCR methodology described here may be an effective tool for application in systematic studies using plant and fungal material preserved in herbaria.

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A systematic study of *Acacia calamifolia s.l.*, with special emphasis on *A. euthycarpa* in Victoria

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Abstract

Taxonomic revision of the *Acacia calamifolia* Sweet *ex* Lindl. complex shows that *A. euthycarpa* (J.M.Black) J.M.Black is distinct, and that the two species have allopatric distributions. Typical *A. calamifolia* has moniliform legumes, narrowly linear phyllodes, united sepals, and an elongated funicle half-encircling the seed. It occurs in New South Wales, and in the Flinders Ranges and North Mount Lofty Ranges of South Australia. *Acacia euthycarpa* has linear legumes, narrowly linear to oblanceolate phyllodes, free to united sepals, and a longer funicle that entirely or nearly entirely encircles the seed. It occurs in Victoria, on the Eyre Peninsula, Kangaroo Island, South Mount Lofty Ranges, and the Murray Lands of South Australia. Multigroup discriminant function analysis of *A. euthycarpa* using phyllode characters resolved two subspecies. *Acacia euthycarpa* subsp. *euthycarpa* has narrowly linear phyllodes. *Acacia euthycarpa* subsp. *oblanceolata* Stephen H. Wright, is newly described to accommodate populations from only two localities in Victoria and three in South Australia which have narrowly oblanceolate to oblanceolate phyllodes. A key to distinguish the new subspecies and the morphologically similar taxa is presented.

Introduction

Acacia calamifolia Sweet *ex* Lindl., as currently understood, has a disjunct distribution throughout much of south-eastern Australian in semi-arid regions with a mean annual rainfall of 150–550 mm. It occurs on a range of soils in open scrub or woodland/open woodland communities and on rocky outcrops. *Acacia calamifolia s.l.* is generally a rounded to Y-shaped, multistemmed shrub 2–4 m high, but occasionally grows to a small, single-stemmed tree to 10 m high in parts of west-central Victoria. It generally has narrow phyllodes 1–5 mm wide, with four main veins (one main vein per face) and a distinctive, curved tip. It has legumes that are linear to moniliform, and an elongated funicle that half to entirely encircles the seed.

Acacia calamifolia var. *euthycarpa* J.M.Black (Black 1923) was distinguished from *A. calamifolia* var. *calamifolia* by its linear legume. Later, when elevating the taxon in rank to *A. euthycarpa* (J.M.Black) J.M.Black, Black (1945) again made the comparison in shape of the legume, but added that the phyllode of *A. euthycarpa* had three nerves. In spite of these differences, the name has long been considered synonymous with *A. calamifolia* (e.g. Whibley 1986; Entwisle *et al.* 1996; Maslin 2001). The complex that includes *A. calamifolia* and *A. euthycarpa* has been considered extremely variable (Whibley 1986; Entwisle *et al.* 1996).

A variant of *A. calamifolia* with broad, oblanceolate phyllodes occurs from South Australia to west-central Victoria (Willis 1972; Entwisle *et al.* 1996). This variant differs from typical *A. calamifolia* not only in size and shape of phyllodes, but also by habit, often becoming a substantial tree to 10 m, and with a funicle almost entirely encircling the seed (Willis 1972). These populations have often been erroneously known as *A. microcarpa* F.Muell. var. *linearis* J.M.Black (e.g. Whibley 1986).

A taxonomic revision of *A. calamifolia s.l.* is given in the following. A morphometric analysis of phyllodes clarifies the taxonomy of broad and narrow phyllode forms in Victoria and South Australia, and supports the recognition of two distinct species, as well as a new subspecies of *A. euthycarpa.*

Methods

Ten populations were sampled in the field for a multivariate analysis of phyllode characters, which are the most variable features of these populations. Legumes and seeds were not available for a quantitative comparison of populations, but were described from both field observations and from herbarium material. Originally flower characters were also measured, but not completed, as they exhibit almost no variation in size. Differences in fruit size were not measured for lack of adequate sample size. Field sampling was restricted to Victoria because nearly all of the variation in phyllode characters was represented in populations from that State. Four populations were sampled from the Wimmera and six from west-central Victoria (Fig. 1). Twenty individuals were sampled across the range of each population, subjectively from small populations (less than ± 50 individuals) and along a walk-transect for larger populations.

One typical mature phyllode was selected from each pressed specimen. Only one phyllode per individual was measured since measuring more phyllodes per individual was found to give no better estimate of plant population means (for those characters included in the analysis) and no less variances. The following phyllode meristic characters were scored: total length, length of pulvinus, width at widest point, and thickness at widest point. Vein number was noted for each population.

Multigroup discriminant function analysis (MDA) was chosen to analyse the phyllode characters because it is useful for comparing variability within and between populations. This analysis uses weighted predictor variables (i.e., weighted phyllode characters), in the form of linear functions, to provide the best discrimination between predetermined groups, in this case plant populations (Tabachnick & Fidell 1989). Each individual phyllode within a group obtains a discriminant value for each function based on its values for predictor variables. Individuals may be classified into populations using probabilities based on the dis-

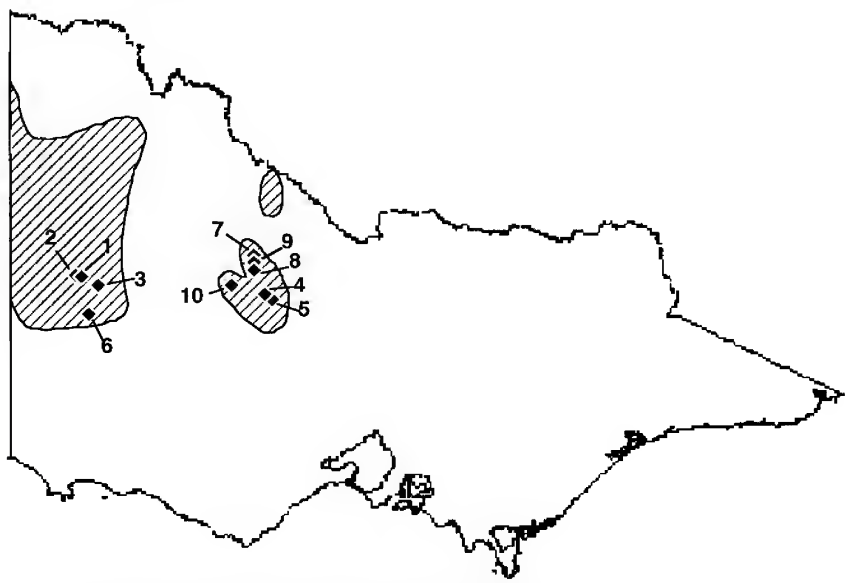

Figure 1. Distribution of *A. calamifolia s.l* in Victoria, with populations sampled for morphometric analysis numbered 1–10.

criminant scores. The analysis also infers the relative contributions of each predictor variable to the classification. Variables were transformed, when appropriate, to meet the assumptions of multivariate normality and equality of variance-covariance matrices.

Following analysis of the wild populations, phyllodes measured from herbarium specimens at MEL were classified by the MDA to determine their relationship with the populations of phyllodes sampled in the field. All statistical methods were performed on a PC computer using the SPSS Windows package (S.P.S.S. 1990).

Results

Two discrete groups were separated by the first discriminant function in the MDA (Fig. 2), which accounted for 96% of the between-population variation in phyllode characters. The transformed variable log (width) was highly correlated (0.80) with this first function, inferring that width was the dominant character for separating populations (Table 1). Of the two groups separated, one had narrow phyllodes, averaging 1.1 mm wide (population 1–8), and the other had broad phyllodes, averaging 3.3 mm wide (populations 9–10 from Wychitella and Yowang Hill, respectively, in west-central Victoria). The means for the untransformed phyllode characters in the narrow and broad groups are shown in Table 2.

Table 1. Correlation between phyllode variables and the first two discriminant functions of the MDA.

	Function 1	Function 2
Log (width)	0.80	0.13
Log (thickness)	-0.23	0.65
Length	-0.06	-0.60
Log (pulvinus)	-0.05	-0.20

Table 2. Means of phyllode characters for the two groups resolved by MDA. Standard Deviations are in parentheses.

	Narrow phyllode group	Broad phyllode group
Width (mm)	1.1 (0.26)	3.3 (0.53)
Thickness (mm)	0.48 (0.07)	0.37 (0.05)
Length (mm)	46.7 (9.1)	40.4 (6.9)
Pulvinus length (mm)	1.53 (0.31	1.65 (0.21)

Within the narrow phyllode group (Fig. 2) populations 1, 2 and 3 from the Little Desert National Park in the Wimmera, and 4 and 5 from near Inglewood in west-central Victoria, have very narrow phyllodes, averaging 0.9 mm. In comparison, populations 4 from Mt Arapiles in the Wimmera, and 7 and 9 from near Wychitella and Wedderburn respectively, in west-central Victoria, are characterised by less narrow phyllodes, averaging 1.3 mm broad. The difference in means of phyllode-width between the populations is not considered useful in delimiting taxa, especially since no other character distinguishes them.

There was no obvious pattern of separation along the second function (Fig. 2), which accounted for only 3% of the between-population variation. The variables length and log (thickness) were the most correlated (-0.60 and 0.65 respectively) with this function (Table 1). By convention, only correlations between variables and functions that were in excess of 0.30 (9% of variance) were interpreted (Tabachnick & Fidell 1989).

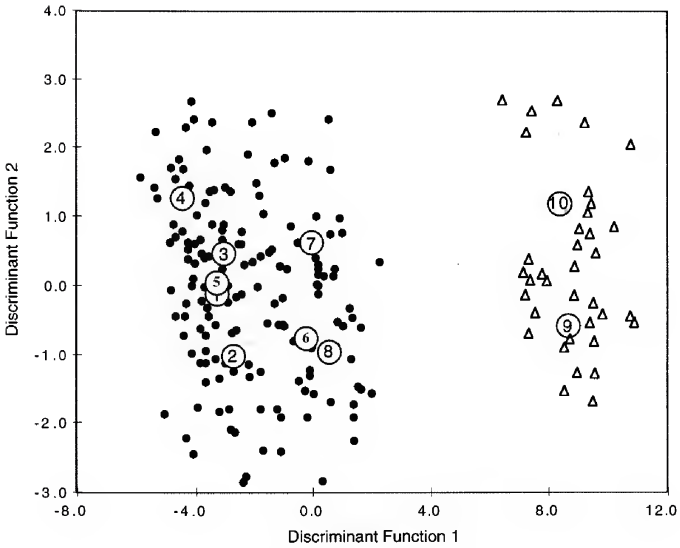

Figure 2. Plot of discriminant function values for phyllodes from individuals from the ten populations sampled in Victoria, and values for the population means. Individuals from populations 1–8, which comprise the narrow-phyllode group, are represented by filled circles; individuals from populations 9–10, which comprise the broad-phyllode group, are represented by open triangles; population means are presented by their population number in shaded circles.

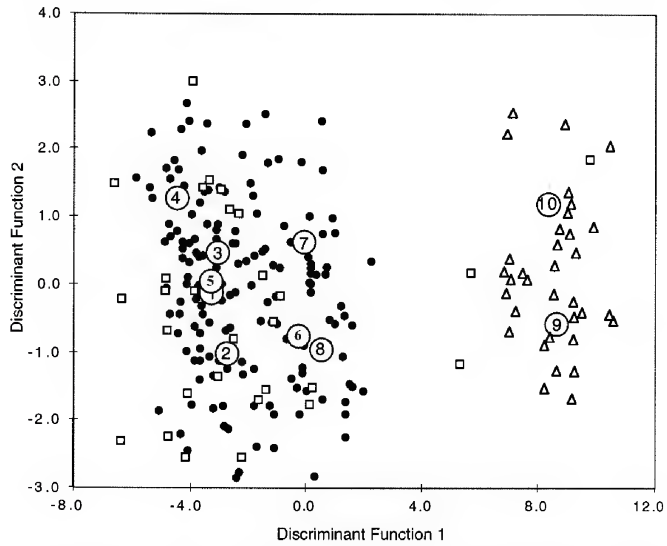

Figure 3. Plot of discriminant function values for phyllodes from individuals from the ten populations sampled in Victoria, values for the population means, and values for phyllodes measured from herbarium specimens from across the geographic range of *Acacia calamifolia s.l.* Individuals from populations 1–8, which comprise the narrow-phyllode group, are represented by filled circles; individuals from populations 9–10, which comprise the broad-phyllode group, are represented by open triangles; population means are presented by their population numbers in shaded circles; individual herbarium specimens are represented by open squares.

Discriminant function values for phyllode variables from herbarium specimens fell within, or near, the range from those sampled in the field in Victoria (Fig. 3).

Discussion

THE SPECIFIC CIRCUMSCRIPTION OF *ACACIA CALAMIFOLIA* AND *A. EUTHYCARPA*

As legume type is generally considered constant with species of *Acacia* (New 1984), the differences in shape between the pods of *A. calamifolia s.s.* and *A. calamifolia* var. *euthycarpa*, in conjunction with differences in seed characters, provides evidence that *A. calamifolia* and *A. euthycarpa* are distinct species.

Furthermore, *A. euthycarpa* generally has two to four capitula per raceme and a rachis length of 1–8 mm. Based on examination of herbarium material, greater variation was detected in *A. calamifolia s.s.*, with two to eight capitula per raceme and a rachis length of 5–25 mm. Sepals are united in *A. calamifolia s.s*, and on specimens of *A. euthycarpa* from Kangaroo Island and South Mount Lofty Ranges. Sepals are free on specimens of *A. euthycarpa* from other areas, suggesting geographic variation in sepal-fusion in this species.

Although nervature of the phyllode was not mentioned in the protologue of *A. calamifolia* var. *euthycarpa* (Black 1923), *A. euthycarpa* was described as having 3-nerved phyllodes ('sed phyllodiis arcte trinervibus,' Black 1945: 310), and on a note on one of the types (AD *97333008*) J.M. Black wrote 'phyllodes... about 3-nved (3-striate) on each face, obscurely 3-nvd.' This character would distinguish it from *A. calamifolia*. In reality this is incorrect; the phyllodes of *A euthycarpa* are four nerved, with one nerve on each face, as in *A. calamifolia*. With age each face of the phyllode becomes faintly striate due to dessication. Care must be taken to distinguish true nerves from striations caused by long-term drying.

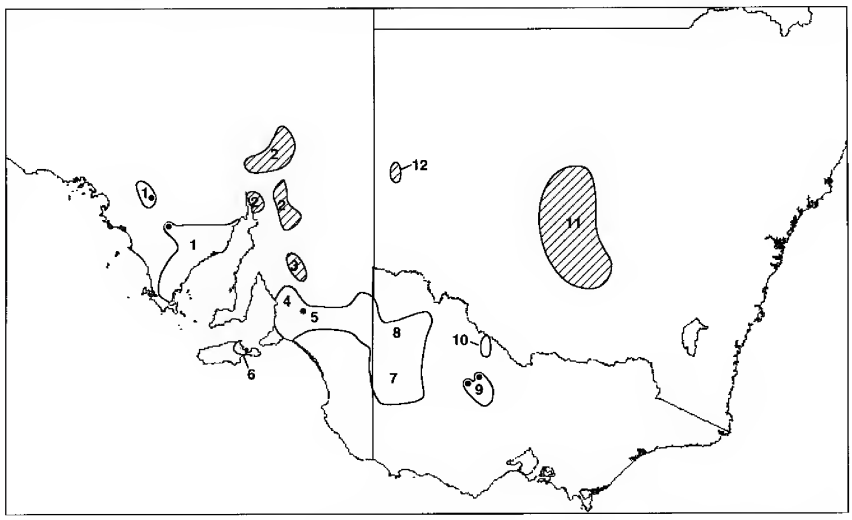

Figure 4. Distribution map of taxa resolved within the *Acacia calamifolia* species complex, showing geographical regions. Distribution of *A. calamifolia s. s.* is shown by hatched areas; distribution of *A. euthycarpa* subsp. *euthycarpa* is shown by shaded areas; localities of *A. euthycarpa* subsp. *oblanceolata* is shown by filled circles. Geographical regions are numbered; Eyre Peninsula (1); Flinders Ranges (2); North Mount Lofty Ranges (3); South Mount Lofty Ranges (4); Murray Lands (5); Kangaroo Island (6); Wimmera (7); Mallee (8); west-central Victoria (9); Swan Hill district (10); Griffith-Cobar region (11); Broken Hill district (12).

Acacia calamifolia s.s. and *A. euthycarpa* have allopatric distributions. *Acacia calamifolia s.s.* occurs in New South Wales, the Flinders Ranges and the North Mount Lofty Ranges of South Australia (Fig. 4). It grows on shallow powdery calcareous loams, grey-brown calcareous earths, and red duplex soils. *Acacia euthycarpa* occurs in Victoria, on the Eyre Peninsula, Kangaroo Island, South Mount Lofty Ranges, and the Murray Lands of South Australia (Fig. 4). It grows on bleached to brownish sands, grey-brown calcareous earths, and mottled-yellow to red duplex soils. Both species grow in scrub or as an understorey species in woodland or open woodland, often on rocky sites. They both grow in a range of rainfall conditions from 150–500 mm mean annual rainfall (arid to moderately arid conditions).

PHYLLODE FORMS IN VICTORIA – TWO SUBSPECIES OF *A. EUTHYCARPA*

The MDA distinguished two forms within *A. euthycarpa*: the broad-phyllode and narrow-phyllode forms. *Acacia euthycarpa* subsp. *euthycarpa* is referrable to the narrow-phyllode form, while the name *A. euthycarpa* subsp. *oblanceolata* is proposed for the broad-phyllode form. The mean phyllode width of *A. euthycarpa* subsp. *oblanceolata* (3.3 mm) is about three times that of *A. euthycarpa* subsp. *euthycarpa* (1.1 mm). There are also lesser differences in the means of the other characters, with *A. euthycarpa* subsp. *oblanceolata* tending to have shorter, thinner phyllodes with longer pulvini and a shorter distance to the widest point. Some of the important differences are illustrated in Figure 5. *Acacia euthycarpa* subsp. *oblanceolata* is geographically widespread over a range of approximately 850 km, but is known only from two localities in Victoria.

It should be pointed out that *A. euthycarpa* is much more common in Victoria than *A. calamifolia* (*A. calamifolia* is known to us only from few, very old collections), and that the description of the latter provided by Willis (1972) applies to *A. euthycarpa.*

The phyllodes of *A.* × *grayana* are very similar to those of *A. euthycarpa* subsp. *oblanceolata*, and the two taxa are difficult to tell apart in flower. However, *A.* × *grayana* has submoniliform fruit as well as pubescent new shoots and peduncles (Entwisle *et al.* 1996).

Acacia euthycarpa subsp. *oblanceolata* grows on grey-brown calcareous earths, mottled-yellow to red duplex soils, and sometimes sand. It occurs in areas of mean annual rainfall ranging between 300 and 500 mm, suggesting that it has a lower tolerance to arid conditions than *A. euthycarpa* subsp. *euthycarpa* (range 150–500 mm). Localities of *A. euthycarpa* subsp. *oblanceolata* are on the periphery of the distribution of *A. euthycarpa* subsp. *euthycarpa*, which may suggest a different ecological preference, or it may be a pattern associated with agricultural clearing.

Key to taxa

1. Legumes (submoniliform to) moniliform; seed 6–9 mm long, the funicle encircling the seed by about 1/2 the circumference; phyllodes 0.7–4.5 mm wide, if 2 mm wide or wider, then new shoots and peduncles subglabrous to covered in hairs
   2. Phyllodes 0.7–1.2 mm wide ..... **1. *Acacia calamifolia***
   2. Phyllodes 2–4.5 mm wide ..... **2. *A.* × *grayana***
1. Legumes linear; seed 4–6 mm long, the funicle entirely encircling the seed; phyllodes 0.7–6.0 mm wide, if 2.0 mm or wider, the new shoots glabrous to glabrate
   3. Phyllodes 0.7–2.0 mm wide, narrowly linear ..... **3A. *A. euthycarpa* subsp. *euthycarpa***
   3. Phyllodes 2.5–6 mm wide, narrowly oblanceolate ..... **3B. *A. euthycarpa* subsp. *oblanceolata***

1. *Acacia calamifolia* Sweet *ex* Lindl., *Bot. Reg.* 10, t. 389 (1824). *Type citation*: 'brought by Mr John Richardson, to Mr. Colvill, from the south-west interior of New Holland." Type not seen, descripition ('*Legumina arcuata articulata*…') and illustration decisive.

Acacia pulverulenta A. Cunn. *ex* Benth., *London J. Bot.* 1: 342 (1842), *nom. illeg., non*

A. pulverulenta Schltdl. (1838). *Syntypes*: interior of New Holland [between the Loddon R. and Pyramid Hill, Vic.], 8 July 1836, *T.S. Mitchell "230"*; Mt Flinders [one of the peaks adjacent to L. Brewster which is c. 10 km S of Lachlan R. and c. 130 km N of Griffith, N.S.W.], June 1817, *A. Cunningham 403* (not found). Neither of these syntypes of *A. pulverulenta* were located at K, and the Cunningham collections could not be found at MEL. The only syntype seen by us is the one collected on Mitchell's journey, with the number '230.'

Shrub 2–4 m high, plants glabrous throughout. *Phyllodes* narrowly linear, 4–10 cm long, 0.7–1.2 mm wide, 0.4–1.0 mm thick, terete to flat, green to grey-green, sometimes scurfy, shortly acuminate with delicate, curved apex; main longitudinal veins four in all, not prominent and the mid-veins often somewhat impressed; pulvinus 1–2 mm long; the small obscure gland inserted 1–10 mm above pulvinus. *Inflorescences* of simple axillary capitula, or of 2–8(–14)-headed racemes, rachis 5–25(–40) mm long, peduncles 4–10 mm long; the capitula with 25–40 golden yellow flowers, these 5-merous; calyx-lobes all united, corolla-lobes all free. Pods moniliform, woody to coriaceous, wrinkled, straight, curved or twisted, to 16 cm long, 3–6 mm wide, grey to brown, ± glaucous; seeds longitudinal in pod, oblong to elliptic in outline, 6–9 mm long, 2.5–4.5 mm wide, dull to slightly shiny, dark brown to black, the filiform funicle mostly 1-folded, encircling half of seed, aril clavate.

Habitat and Distribution: Found mostly in scrub and sometimes in woodland or open woodland, occasionally on rocky sites; mostly on shallow powdery calcareous loams, grey-brown calcareous earths, or red duplex soils. Flinders Ranges (SA), North Mount Lofty Ranges (SA), Griffith-Cobar region (NSW) and near Broken Hill (NSW).

Phenology: Flowers mostly September to November, but often found sparsely flowering throughout the year.

Selected specimens examined: SOUTH AUSTRALIA: Flinders Ranges, near Wilpena Pound, ca. 45 km NNE of Hawker, *R. Hill 6*, 14 Jul 1955 (MEL); Northern Flinders Range, Northern Chace's Range, rocky banks of Anginoonor Creek, ca. 30 km NE of Hawker, *D.N.Kraehenbuehl 310*, 12 June 1961 (MEL); Oraparinna Natl Park, near headquarters, *J.Z.Weber 2648*, 19 Sep 1971 (AD, MEL); Morgan to Eudunda road, 2.8 km WSW of Sutherlands, NW side of road, *F.E.Davies 1391*, 22 Nov 1989 (AD, MEL).

2. ***Acacia* × *grayana*** J.H.Willis, *Victorian Naturalist* 73: 155 (1957). *Type citation*: 'Wraigworm Parish, south of Kiata and about 14 miles east of Dimboola. *A.J.Gray s.n.*, 9.IX.1951.' (holotype MEL *1500364*, also on the sheet, a sterile specimen of *A.* × *grayana*, same locality as type, leg. 3/1951; and a fruiting specimen collected from a tree raised from seed off the type at Wail Nursery, leg. 3/11/1953; also a specimen of *A. hilliana* and a specimen of *A. euthycarpa* subsp. *oblanceolata*, placed there 'for comparison' (J.H.Willis).)

Acacia microcarpa F.Muell. var. *linearis* J.M.Black, *Flora of South Australia* 4: 687 (1920). *Type citation*: 'Near Monarto South' (holotype AD).

Shrub or small *tree* to 3 m tall, glabrous save for the glabrate to densely appressed hairy peduncles and new shoots. *Phyllodes* elleptic-oblanceolate to broadly oblanceolate, 2.5–5.75 mm long, 2.0–4.5 mm wide, 0.25–0.8 mm thick, flat, dull green to brown-green, acuminate with a curved apex; longitudinal nerves four, one on each broad face of the phyllodes, the two lateral ones appearing as marginal nerves, often becoming more or less irregularly striate with dessication; pulvinus 0.75–1.25 mm , the obscure to subprominent gland inserted 4–9 mm above pulvinus. *Inflorescences* of solitary capitula or short racemes of 2–5-capitula; rachis 2–7 mm long, peduncles 4–12 mm long; the capitula with 20–35 flowers, these 5-merous; calyx-lobes free to the base, corolla-lobes free. *Pods* (sub)moniliform, to 7(–7.5) cm long, 6 mm wide; seeds longitudinal in pod, ± 6 mm long, the funicle encircling about half the seed.

Habitat and Distribution: In sandy soils, often where moist, and often with *Callitris*.

Known from north-western Victoria near Wyperfeld National Park and the Little Desert National Park.

Phenology: Blooming as early as June, but mostly through November.

Selected specimens examined: VICTORIA: Wimmera. Winiam, S of Nhill. *I.O.Maroske s.n.*, 11 Oct 1992 (MEL); west of Yarto, just within the eastern boundary of Wyperfeld Nat'l Park, *I.O.Maroske s.n.*, 25 Jun 1960 (MEL); 1/2 mile [0.8 km south of Kiata Store Kiata, *E.M.Canning 2981*, 11 Oct 1969 (CANB, MEL).

3. *Acacia euthycarpa* (J.M.Black) J.M.Black, *Trans. & Proc. Roy. Soc. South Australia* 69: 310 (1945) (Typus infra sub subsp. *euthycarpa* indicatur).

Shrub 2–4 m high, or occasionally a small *tree* to 10 m high; plants glabrous throughout. *Phyllodes* narrowly linear, narrowly oblanceolate to oblanceolate, 3–8 cm long, 0.7–6.0 mm wide, 0.3–0.8 mm thick, terete to flat, green to grey-green, sometimes scurfy, shortly acuminate with delicate, curved apex; main longitudinal veins four in all (one per face), not prominent and the mid-veins often somewhat impressed; pulvinus 1–3 mm long; the small obscure gland inserted 0–7 mm above pulvinus. *Inflorescences* of simple axillary capitula, or of 2–4(–6)-headed racemes; rachis 1–8(–14) mm long, peduncles 4–10 mm long; the capitula with 25–40 golden yellow flowers, these 5-merous; calyx-lobes free or united, corolla-lobes all free. *Pods* linear, coriaceous to crustaceous, smooth or nearly smooth, straight, curved or twisted, to 16 cm long, 3–6 mm wide, brown; seeds longitudinal in pod, oblong to elliptic in outline, 4–6 mm long, 2.5–4 mm wide, dull to slightly shiny, dark brown to black, the filiform funicle mostly 2–3-folded, entirely encircling the seed, aril clavate.

3A. *Acacia euthycarpa* (J.M.Black) J.M.Black **subspecies *euthycarpa*.** *Acacia calamifolia* var. *euthycarpa* J.M.Black, *Trans. & Proc. Roy. Soc. South Australia* 47: 269 (1923), *s. s. Type citation*: 'Southern districts: Yorke [sic] Peninsula, Kangaroo Island, Eyre Peninsula.' (lectotype, here designated, AD *97333008*, the fruiting specimen on the right-hand side of the sheet labelled 'Barossa Ranges, Nov. 1912;' syntype, also on AD *97333008*: labelled 'Arno Bay [Eyre Peninsula],' sterile spec.; but excluding a specimen labelled 'Nurioota' which is part of the Mount Lofty Ranges, not the York Peninsula; AD *97333011*, labelled 'Port Lincoln [Eyre Peninsula]'; AD *973330006*).

Phyllodes narrowly linear, 3–8 cm long, 0.7–2.0 mm wide, 0.3–0.8 mm thick, terete to flat; the gland inserted 0–7 mm above pulvinus.

Habitat and Distribution: Found mostly in scrub, woodland or open woodland, occasionally on rocky sites; mostly on bleached to brownish sands, grey-brown calcareous earths, and mottled-yellow to red duplex soils. Common in west-central Victoria, western Victoria, Kangaroo Island (SA), Eyre Peninsula (SA), South Mount Lofty Ranges (SA) and the Murray Lands (SA).

Phenology: Flowers mostly August to November.

Selected specimens examined: VICTORIA: 8.2 km S of Wychitella on Wychitella-Wedderbrun Road, *S. Wright 13 and J. Grimes*, 7 Feb 1998 (AD, BRI, CANB, MEL, MELU, PERTH); State Park west of Inglewood, corner of Barry Rock Road and the road from the Logan-Inglewood Road to Melville Caves, *S. Wright 14 and J. Grimes*, 7 Feb 1998 (AD, CANB, MEL, MELU, PERTH); 8.6 km west of Inglewood on the Logan-Ingelwood road, *S. Wright 15 and J. Grimes,* 7 Feb 1998 (MEL); 5.5 km SE of Wedderburn, Calder Highway between Wedderburn and Inglewood, *J. Connock 348*, 12 Sep 1992 (AD, MEL); Summit of Mt Arapiles, Mount Arapiles State Park, *P.G. Abeel 525* and *C. Herscovitch*, 17 Dec 1986; 21.9 km N of Kaniva on the Broughton Road, *J. Grimes 3434* and *B. Meurer-Grimes*, 13 Aug 1996 (AD, BH, CANB, MEL, NSW, NY); Wimmera. Lawlot Range, on Western Highway, *P.C. Jobson 3704*, 27 Aug 1995 (AD, BRI, CANB, MEL, NSW).

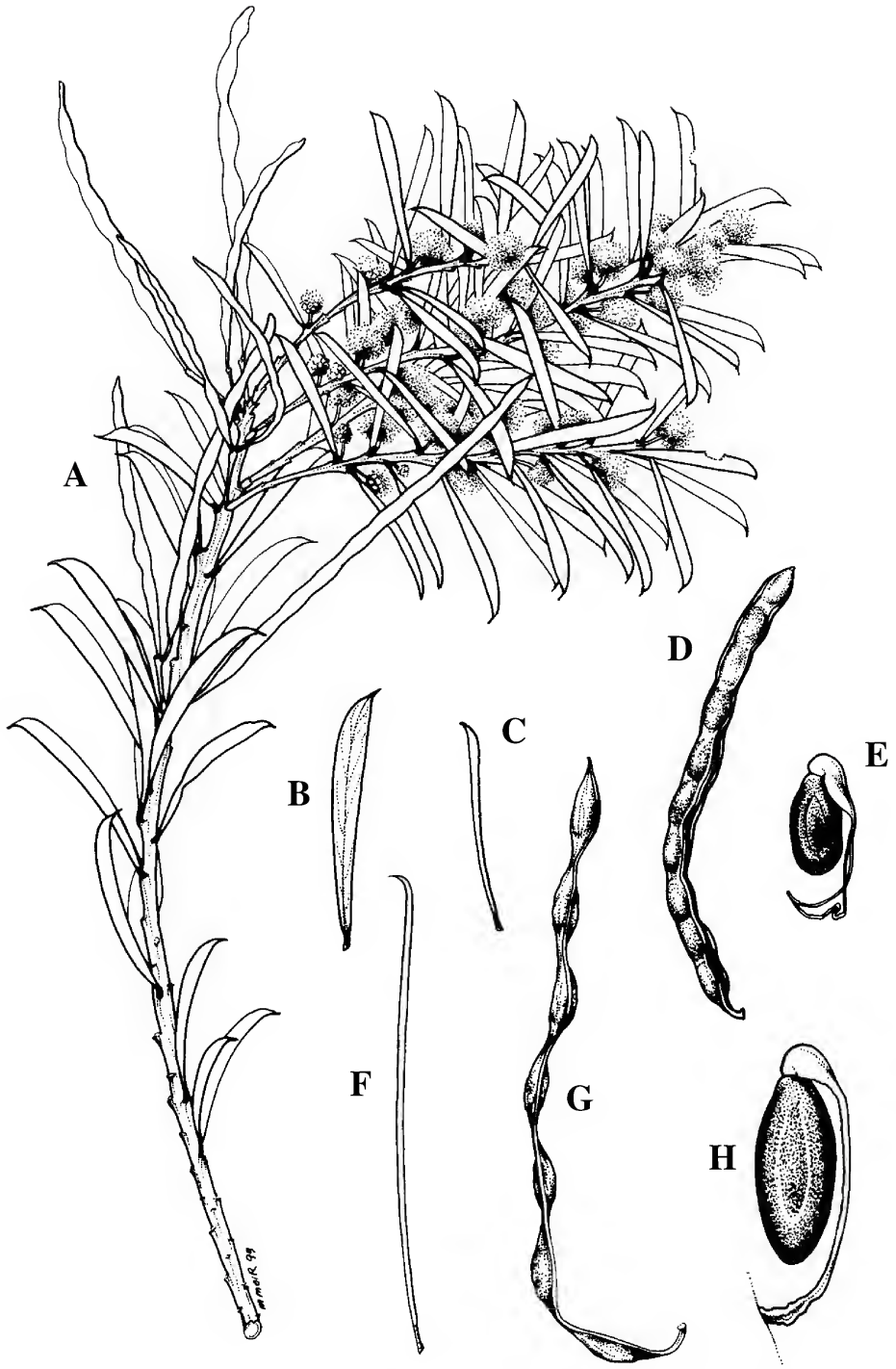

Figure 5. *Acacia euthycarpa* subsp. *oblanceolata*, and comparative illustrations of *A. euthycarpa* subsp. *euthycarpa*, and *A. calamifolia*. **A–B.** *A. euthycarpa* subsp. *oblanceolata*. **A** habit × 0.7; **B** phyllode × 1 (A from *S. Wright 18*; B from *M.G. Corrick 5383*); **C–E.** *A. euthycarpa* subsp. *euthycarpa*. **C** phyllode × 1; **D** fruit × 1 and **E** seed × 3 (from *Maroske s.n.,* Dec. 1966). *Acacia calamifolia* **F–H**. **F** phyllode × 1; **G** fruit × 1; **H** seed × 3 (from *R. Filson 3488*).

3B. *Acacia euthycarpa* subspecies *oblanceolata* Stephen H. Wright, *subsp. nov.*

a *A calamifolio* legumine lineari diversa; a *A. euthycarpa* subsp. *euthycarpa* phyllodio latiore (2.5–6 nec 0.7–2.0 mm) diversa (Fig. 5a–e)

Typus: VICTORIA, Yowang Hill, northeast of St Arnaud, 36°29'S 143° 22'E, *S. Wright 18* (holotype MEL *2058890*; isotypes AD, BRI, CANB, MELU, NSW, PERTH).

Phyllodes narrowly oblanceolate to oblanceolate, 3–6 cm long, 2.5–6.0 mm wide, 0.3–0.5 mm thick, flat, the gland inserted 0–5 mm above pulvinus.

Habitat and Distribution: Found in scrub or open woodland, often on rocky sites; on grey-brown calcareous earths and mottled-yellow to red duplex soils, sometimes on sand. Rare, occurring at only six known localities: Wychitella Flora and Fauna Reserve and Yowang Hill (west-central Victoria), Gawler Ranges and near Kima Eyre Peninsula (SA) and near Murray Bridge (the Murray Lands SA). The specimen near Murray Bridge was collected in 1848 and this subspecies may no longer occur there.

Phenology: Flowers mostly August to October.

Remarks: Appears similar to *A.* × *grayana*, a hybrid between *A. euthycarpa* subsp. *euthycarpa* and *A. brachybotrya*, occurring near Kiata in the Little Desert, but may be distinguished from *A.* × *grayana* by its glabrous new shoots and peduncles and rectilinear pod.

Conservation status: A ROTAP code of 3R is proposed for Victoria. The subspecies is fairly widespread but occurs in relatively isolated populations. Though on both crown land and in reserves, in many places it is threatened by sheep grazing.

Selected specimens examined: SOUTH AUSTRALIA, Eyre Peninsula: S side of Eyre Highway, 30 km W of Port Augusta, *P.C. Jobson 2726*, 28 Oct 1993 (AD, MEL); c. 65 km W of Kimba, c. 27 km east of Kyancutta, *T.R.N. Lothian 5409*, 10 Oct 1986 (BRI, MEL, PERTH); 2.9 km south of Wychitella, on Wychitella-Wedderburn road, *S. Wright 12 and J. Grimes*, 7 Feb 1998 (CANB, MEL *2058891*, MELU, PERTH).

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Muelleria 16: 65–70 (2002)

Agyrium Fr., *Bryophagus* Nitschke ex Arnold and *Racodium* Fr., lichen genera previously unrecorded for Australia

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Abstract

Agyrium rufum (Pers.) Fr. (Agyriaceae), *Bryophagus minutissima* (Vězda) D. Hawksw. (Gyalectaceae) and *Racodium rupestre* Pers. (*incertae sedis*) are recorded from Tasmania, representing the first reports of these lichen genera for Australia. Morphological and anatomical data, as well as information on the distribution and ecology of each species is presented.

Introduction

As a result of ongoing research on the Australian lichen flora, new records for the whole continent and especially for individual States continue to be reported; for example, see the ongoing series of papers in the journal *Australasian Lichenology*. However, most such records concern species, and new generic records are relatively infrequent. Studies on crustose lichens from Tasmania, and comparisons with authentic reference material, mainly from the Northern Hemisphere, have led to the identification of three genera previously unrecorded for Australia. Two, *Agyrium* Fr. and *Racodium* Fr., are cool temperate taxa and further demonstrate the strong floristic links that exist between Tasmania and the temperate, oceanic regions of the Northern Hemisphere. The third, *Bryophagus* Nitschke ex Arnold, has a more scattered distribution.

Methods

The work is based on the author's collections from Tasmania, held in the Tasmanian Herbarium (HO) and on comparative material in the Natural History Museum, London (BM), the Royal Botanic Garden, Edinburgh (E) and the Karl-Franzens-Universität, Graz (GZU). Observations and measurements of apothecial tissues and ascospores were made on hand-cut sections and squashes mounted in dilute KOH solution.

Agyrium Fr.

The genus *Agyrium* is characterised by a poorly developed, immersed thallus, apothecial ascomata with a reduced annular exciple, richly branched paraphyses, eight-spored, *Trapelia*-type asci and simple, non-halonate, thin-walled, ellipsoid ascospores (Dennis 1981; Purvis & James 1992a; Lumbsch 1997). Although non-lichenised in the strictest sense, the mycelial hyphae are frequently associated with and penetrate green coccoid algae, especially in the vicinity of the apothecia, a condition described by Lumbsch (1997) as 'facultative parasitism'. Thus *Agyrium* superficially looks like a lichen, and its closest relatives are all lichens, including, in the Australian lichen flora, *Lithographa* Nyl., *Placopsis* (Nyl.) Lindsay, *Placynthiella* Elenkin, *Rimularia* Nyl., *Trapelia* M.Choisy, *Trapeliopsis* Hertel & G.Schneider and *Xylographa* (Fr.) Fr. These and some additional genera not known from Australia comprise the family Agyriaceae and are discussed extensively in the comprehensive paper of Lumbsch (1997).

Although several taxa have been included in *Agyrium* in the past, many of these have been transferred formally to unambiguously non-lichenised, unrelated genera. Only one species, *A rufum* (Pers.) Fr. is currently recognised in the Northern Hemisphere (see Dennis 1981 and Purvis & James 1992a for descriptions) and occurs widely in Europe,

mainly on dead wood. This species is here recorded from Tasmania, representing the first record of the genus for Australasia. Two additional species of *Agyrium* are known from southern South America: *A. antarcticum* Rehm, reported from rotting *Nothofagus*, and *A. chilense* Speg., from rotting *Lobelia*.

Agyrium rufum (Pers.) Fr., *Systema Mycologicum* 2: 232 (1822); *Stictis rufa* Pers., *Observationes Mycologicae* 2: 74 (1799). Type: *n.v.*

Thallus immersed to absent, usually defined by pale, bleached patches of the substratum. Algal cells occasional, globose to broadly ellipsoid, 4–7 x 4–6 µm, mostly occurring around or beneath the apothecia. Apothecia scattered, pale orange to orange-red to red-brown, waxy or matt, convex, irregularly roundish, sometimes rather wrinkled or contorted, immarginate, 0.2–0.5 mm wide, 0.12–0.2 mm thick. Exciple (in section) very reduced, 20–40 µm thick, pale to deep orange-brown, K+ orange-red to red, sometimes fleetingly or soon becoming ± persistent pale yellow. Subhymenial tissues poorly differentiated, sometimes also with irregular patches pigmented as above. Hymenium 70–80 µm thick, intensely I+ blue, with an orange-brown pigmented epithecial layer c. 10 µm thick, sometimes uneven and extending deeper into the hymenium; reaction in K as above. Asci broadly clavate, 60–75 × 12–22 µm, of the typical *Trapelia*-type: outer wall amyloid; apex broadly rounded; tholus thick and well-developed, with a thin amyloid cap and weakly amyloid flanks, ± non-amyloid in the remainder; ascoplasm truncate to concave at the apex, without an ocular chamber (Fig. 1A; also see Rambold & Triebel 1990). Paraphyses richly branched, c. 1 µm thick, with somewhat swollen apices 1.5–2 µm thick. Ascospores 12–8 × (5–)6–8(–9) µm, broadly ellipsoid or egg-shaped, hyaline at first, becoming grey to deep brown when old, typically with one or several vacuoles.

Remarks: The above description is based on the Tasmanian specimens cited below. Their ascospores are somewhat larger than commonly seen in European material, although there is no major difference between the two populations. Brown spores are quite common in the Tasmanian material but were rarely seen in specimens from Europe. Similarly the pigmentation of apothecial tissues (and consequent K reaction) is more intense in Tasmanian specimens. These differences are likely to relate to the age of the apothecia and the degree of exposure of the microhabitat.

All Tasmanian specimens fall within the concept of *A. rufum* as accepted by Northern Hemisphere workers, but also display some critical morphological and ecological differences between them. One specimen is from alpine heathland where *Cetraria australiensis* W.A.Weber ex Kärn. and species of *Cladia* Nyl., *Cladonia* Hill ex P.Browne and *Cladina* Nyl. predominate. It grew on the dead, decorticated, bleached twigs of an unknown shrub, possibly a member of the Epacridaceae, associated with several other unusual crustose lichens including species of *Xylographa* (Fr.) Fr. and *Japewia* Tønsb. It has rather small, orange-red, waxy apothecia and is identical to European material, especially that which occurs on dead, bleached *Calluna* (Ericaceae) stems. Specimens from decorticated, bleached lignum of eucalypt logs in wet forests are virtually identical to European specimens from conifer lignum. The discovery of *Agyrium* in this habitat, especially as a pioneer in post-logging regeneration in forestry coupes, suggests the species may be quite widespread in Tasmania and has been previously overlooked. The specimen from cool temperate rainforest, growing on the bark of a very old, dry trunk of a mature *Nothofagus cunninghamii* tree, is unusual: the apothecia are a very deep reddish brown, and some of the younger apothecia have a scattered orange pruina; both young and old fruiting bodies are deeply pigmented within, and the K+ red reaction is very intense and persistent, especially in the excipulum; brown spores predominate in this specimen. These characters are rather extreme in the context of all the material studied, but until

more material is found, the specimen is included within *A. rufum*. It is rather similar to British material of *Lecidea grumosa* Leight. (=*A. rufum*) which is also from bark and is rather intensely pigmented within.

Authentic material of the South American species could not be located for comparison, but on the basis of descriptions both differ clearly from *A. rufum* on spore size alone. In *A. antarcticum* the spores are 10–12 × 8–10 µm and clearly broader than those of *A. rufum* (Rehm 1899), whereas in *A. chilense* they are 10–11 × 2–2.5 µm (Saccardo & Trotter 1913) and narrower.

Australian specimens examined: **Tasmania:** Arthur River, 250 m altitude, on *Nothofagus cunninghamii* in rainforest, 13.ii.1982, *G Kantvilas* s.n. (HO); summit of Wild Dog Tier, 41°47'S 146°35'E, 1390 m altitude, on dead wood in alpine heathland, 11.iii.2001, *G. Kantvilas* 375/01 (BM, HO); track to Mother Cummings Peak, 41°41'S 146°32'E, 1150 m altitude, on decorticated eucalypt wood in subalpine scrub, 3.iii.2002, *G. Kantvilas* 147/02 (HO); west of Tahune Bridge, 'Small Coupe', 43°06'S 146°42'E, 100 m altitude, on decorticated bleached eucalypt log in regenerating logging coupe, 19.ii.2002, *G. Kantvilas* 171/02 (GZU, HO).

Selected comparative material examined: **United Kingdom:** West Ross (V.C. 105), Kinlochewe, Beinn Eighe NNR, Coille na Glas-leitir, c. 100 m altitude, on lignum of fallen, decorticate *Pinus*, 12.iv.2001, *B.J. and A.M. Coppins* s.n. (HO); East Ross (V.C. 106), Amat Forest, on *Betula* lignum, 28.v.1975, *B.J. Coppins 2224 & F. Rose* (E); Perthshire, Tulloch, near Killiecrankie, on decorticate *Calluna* twigs in burn-out, 24.vi.1972, *R. Watling* (E); Easterness (V.C. 96), Abernathy Forest, on decorticated branch of *Pinus*, 1250 feet altitude, 24.v.1976, *B.J. Coppins 3162 and L. Tibell* (E); South Hants (V.C. 11), New Forest, Brockenhurst Woods, Bakers Copse, on decorticated *Fraxinus*, 31.i.1982, *P.W. James* (BM). **Ireland:** Galway, Ballynahinch, on pine bark, ii.1877, *Larbalestier* (BM). **Austria:** Steiermark, Rücken der Aflenzer Staritzen NE über Aflenz, 1700-1800 m altitude,

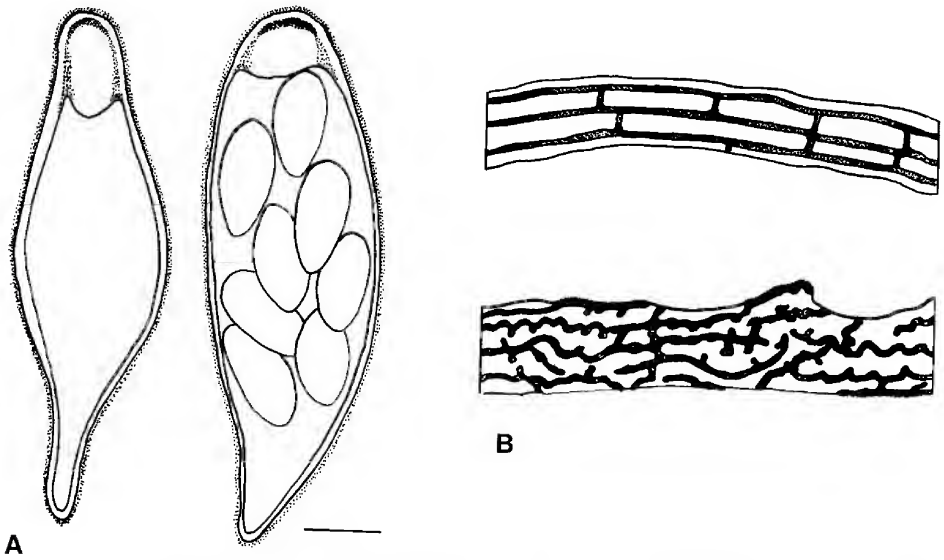

Figure 1. A: Asci and ascospores of *Agyrium rufum* with amyloid parts stippled; B: portion of thallus filaments of *Racodium rupestre* (above) and *Cystocoleus ebeneus* (below), showing the different arrangement of the dark fungal hyphae on filaments of the alga *Trentepohlia*. Scales: A= 10 µm; B= 20 µm.

2.ix.1984, *I. Brodo and J.Poelt* (GZU); Salzburg, Pinzgau, Nationalpark Hohe Tauern, Wildgerlostal, zwischen Gasthaus Finkau (1420 m) und Trisslalm (1583 m), 23.vii.1992, *C. Scheuer* (GZU).

***Bryophagus* Nitschke ex Arnold**

Bryophagus is a genus of three species currently included in the family Gyalectaceae: *B. gloeocapsa* Nitschke ex Arnold from Europe, *B. similis* (Vězda) Kalb, from tropical America, and *B. minutissima* (Vězda) D. Hawksw. from New Guinea. This last species is here recorded from Tasmania, representing the first report of the genus for Australia. Accounts of the genus are provided by Vězda (1966, 1973) and Purvis and James (1992b). In the past, nomenclatural problems caused all these species to be included in the genus *Gloeolecta* Lettau, but this name is now regarded as a synonym of *Bryophagus* (Hawksworth *et al.* 1980).

Bryophagus is superficially similar to several other genera of tiny, inconspicuous crustose lichens, especially to *Gyalecta* Ach. and *Absconditella* Vězda (neither presently known in Tasmania), and *Gyalidea* Lettau (represented in Tasmania by *G. hyalinescens* (Nyl.) Vězda). Taxa in these genera typically have a crustose, sometimes ± gelatinous thallus, small to minute, gyalectoid, pale-coloured to translucent apothecia, elongate, clavate asci typically with only a slightly thickened apex, simple paraphyses and hyaline, frequently 3-septate ascospores. Separation of these genera is not straightforward. In *Gyalecta*, the photobiont is *Trentepohlia* whereas the other genera have a coccoid photobiont; in the case of *Bryophagus*, the photobiont is the distinctive *Gloeocystis* in which the small, ± globose or ellipsoid cells are grouped within a thick gelatinous sheath. Although having asci with an amyloid wall after pretreatment in KOH, the asci of *Absconditella* have a rather prominent, thickened tholus whereas those of *Bryophagus* are only very slightly thickened. *Gyalidea* and *Gyalecta* have asci rather similar to *Bryophagus* but these are entirely non-amyloid. In addition, the ascospores of *Gyalidea* have a thin gelatinous perispore whereas those of *Bryophagus* do not.

Bryophagus minutissima (Vězda) D.Hawksw., in D.E. Shaw, *Microorganisms in Papua New Guinea*: 248 (1984); *Gloeolecta minutissima* Vězda, *Folia Geobot. Phytotax.* 8: 312 (1973). Type: Papua New Guinea: eastern Highlands, Bismarck Ranges, Mt Wilhelm, Imbuku Ridge above Lake Auende, 3450 m, 26.vi.1968, *W.A. Weber & D. McVean* (holotype COLO L48422b, *n.v.*).

Thallus not visible, evident only as necrotic patches over the bryophyte substratum. Photobiont cells globose, 2–3 µ, with a thick gelatinous sheath to c. 5 µm thick. Apothecia scattered over or slightly embedded in the substratum, lecideine, 0.6–1.3 mm wide, hyaline, gyalectoid, with a markedly and persistently concave disc, partly obscured by an incurved, cup-shaped proper margin when young, becoming gaping and excavate when old. Excipulum hyaline, c. 20 µm thick. Hymenium 30–40 µm thick, hyaline, I+ faint yellow-brown before pre-treatment with KOH, persistently I+ pale blue after pre-treatment. Asci cylindrical-clavate, 26–35 × 4–4.5 µm, 8-spored; apex slightly thickened, I-; outer wall I+ blue, somewhat thicker at the apex of the ascus. Paraphyses simple, mostly ± straight, 0.5–0.8 µm thick, with apices very slightly thickened. Ascospores bacilliform, 3-septate, 8–13 × 1 µm. Pycnidia unknown.

Remarks: The Tasmanian specimen is very sparingly fertile and with most apothecia over-mature and with eroded hymenia. Hence, the anatomical data given above are based mainly on the type description by Vězda (1973), supplemented where possible with observations from the Tasmanian material. In Tasmania, this species occurs over a mat of

hepatics on disturbed, sandy soil in an abandoned copper-mining area, a habitat also favoured by the Northern Hemisphere species, *B. gloeocapsa*. *Bryophagus minutissima* is extremely inconspicuous and easily overlooked, especially as the thallus is almost absent and the apothecia vary from small to minute, are ± translucent and very difficult to detect even with a lens. Indeed the best clue to its presence is provided by the death in small circular patches of its bryophyte hosts.

Bryophagus minutissima is well separated from the other species of the genus by its markedly smaller apothecia and ascospores. In *B. gloeocapsa*, the apothecia are 0.2–0.5 mm wide, rather immersed in a glossy, gelatinous thallus, and the spores are 20–30 × 1.5–2 µm. In *B. similis*, the apothecia are 0.3–0.5 mm wide and the spores are 12–15 × 2.5–3 µm.

Australian specimen examined: **Tasmania:** Queenstown, opposite old Mt Lyell Mine Office, 42°05'S 145°33'E, on hepatics over gravelly bank colonised by bryophytes, 200 m altitude, 6.ii.1984, *G. Kantvilas & P.W. James* 191/84 (BM, herb. Vězda, HO).

Racodium Fr.

The genus *Racodium* is an obligately sterile genus of filamentous lichens. In the past, it has also included non-lichenised representatives but, as discussed in detail by Hawksworth (1970), it is correctly based on the lichenised hyphomycete *R. rupestre* Pers. and should be considered monotypic. This species is known from Europe and North America and has recently also been recorded from New Zealand (Wirth 1997).

Racodium rupestre Pers., *Tent. Disp. Meth. Fung.*: 76 (1797). Type: *n.v.*

Thallus filamentous, forming irregularly spreading, black, felt-like patches on the sheltered faces of rocks in cool, damp environments; filaments loosely entangled, 10-20 µm wide. Photobiont *Trentepohlia*, forming long chains of cells. Fungal hyphae c. 1 µm thick, arranged in a parallel manner along the length of the filaments and forming a rectangular, net-like pattern. Apothecia and pycnidia unknown.

Remarks: The Tasmanian specimens accord completely with authentic material from Europe (for description see Dalby 1992). *Racodium rupestre* is superficially very similar to *Cystocoleus ebeneus* (Huds.) Hariot, a lichen which occurs in identical habitats but appears to be far more common and widespread. The two taxa can only be separated by high-power microscopy. *Cystocoleus ebeneus* differs by having fungal hyphae which are of noticeably uneven thickness, arranged irregularly over the surface of the algal filaments (see Fig 1B and Purvis *et al.* 1992).

Racodium rupestre is uncommon in Tasmania although it may well have been overlooked. In contrast, it is significant that the similar *C. ebeneus* is very common and has been very frequently collected. The sheltered, shaded, often overhanging faces of large boulders in generally moister vegetation types are the typical habitat for both species. Both taxa are typically found at higher elevations in open woodland and heathland, although *C. ebeneus* is also known to occur in the lowlands, even on coastal rocks.

Australian specimens examined: **Tasmania:** track to Mother Cummings Peak, 41°41'S 146°32'E, on sheltered faces of large dolerite boulders at scrubby rainforest edge, 1000 m altitude, 3.iii.2002, *G. Kantvilas* 149/02 (HO); Mt Sprent, 42°48'S 145°58'E, on Precambrian rocks in alpine heathland, 1050 m altitude, 17.ii.1987, *G. Kantvilas s.n.* (HO); Mt Arrowsmith, 42°12'S 146°05'E, on rocks, 960 m altitude, 14.xi.1964, *G.C. Bratt* 1778 (HO).

Comparative material examined: **Sweden:** Härjedalen Province, southern slope of Mt Gruvvålen, 62°43'N 12°25'E, on exposed rock in the upper part of the subalpine region, c. 900 m altitude, 1.ix.1970, *R. Santesson* 22567 (*Lichenes Selecti Exsiccati Upsaliensis* 45) (GZU).

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Muelleria 16: 71–79 (2002)

Alexander Clifford Beauglehole OAM (26 August 1920–19 January 2002)

Cliff Beauglehole, the youngest of three sons of Richard and Margaret Beauglehole, was born in Portland in south-west Victoria (A.C. Beauglehole, undated). For the first five years of his life he lived on his parents farm south of the Portland–Bridgewater road in the Parish of Trewalla. In 1926 the family moved to his parents other farm at Gorae West. Cliff's schooling was of short duration as he only attended the tiny Gorae State School, eight kilometres away, for six years. To reach school he rode each day on a pony along rough bush tracks. Possessing a natural curiosity and encouraged by his parents who were both interested in the natural world, it was during these formative years that his interest in natural history was initiated and stimulated. Thus began an interest in the natural world that was a life-long preoccupation.

The Beaugleholes had as family friends the Holmes family at Gorae. Members of the Holmes family were very keen nature lovers who shared freely their knowledge of the bush. By the age of ten Cliff knew and could name scientifically about sixty species of orchids from the district. By the time Cliff left school he had a working knowledge of much of the local flora and fauna, but particularly of orchids and birds. On leaving school Cliff assisted his parents on their mixed farm at Gorae West to which the family had moved.

Orchids were of special interest to Murray Holmes and this interest transmitted itself to Cliff. Together they covered hundreds of kilometres on horse, pushbike and on foot searching for native orchids. He received encouragement also from Mrs. Floss Mellblom, another local orchid enthusiast, who executed coloured illustrations of the local orchid species to enable others to identify them more readily. One of Cliff's prized possessions was a set of illustrations that she presented to him. Encouraged by Murray and Floss, in 1934 at the age of fourteen Cliff began corresponding with and sending specimens for identification to W.H. Nicholls in Melbourne, one of the leading Australian orchid specialists of the time. Nicholls (1934) replied to what was possibly Cliff's first letter to him by stating 'Yes. I know a little about Orchids – no one knows everything.' The following year, on account of illness, Nicholls (1935) wrote to Cliff '.....Would you think me unkind to ask you to write to The Government Botanist (Mr. Rae) and forward all specimens to him for determination. The new 15000 Pound Herbarium is now opened and they have all the facilities there to give you full particulars of all the flora known. You will find them very obliging. .....' Thus began an association with the National Herbarium of Victoria that was to last for over sixty-five years.

Soon after leaving school Cliff started a systematic survey of the flora of the Portland region in his spare time, in the process doubling the number of species recorded for the area. On account of this activity he was christened 'King of the Gorae Forest' (A.C. Beauglehole, undated). By the early 1940s Cliff was corresponding with Rev. H.M.R. Rupp and supplying him with specimens of orchids. The years 1941 and 1942 were especially good seasons for orchids during which he discovered three previously undescribed species. His first paper 'Orchids of the Portland District' was published in the *Victorian Naturalist* 60: 23-25 (1943). In this paper were listed the scientific name, common name, date of first collection and the localities at which each species of orchid was collected. The compilation of species lists was a format that Cliff favoured throughout his career. Rev. Rupp suggested to Cliff that he should study all plants. Cliff readily accepted this challenge and was encouraged by the botanists including Jim Willis at the National Herbarium of Victoria to do so. He extended his botanical explorations in south-west Victoria in search of mosses, liverworts, fungi, lichens, and freshwater and marine algae. Together with Jim Willis he conducted field work in the Portland area, Grampians and elsewhere.

In September 1941 Cliff began to contribute to B E Carthew's column 'Nature Notes' in the 'Portland Observer.' Between 1941 and 1949 Cliff contributed almost monthly to this column, and a substantial proportion of about one hundred articles contain information on Cliff's observations.

In October 1949 Cliff married Hilda Mary Oakley. Life on the farm, which he purchased from his parents, was very busy with potato growing, an apple orchard and dairy cows, especially following the birth of two daughters, Valerie and Yvonne. The change from dairy cows to beef cattle made life a little easier for Cliff to pursue his natural history interests. Hilda always took a keen interest in, and often helped with, Cliff's various projects. Throughout these years on the farm Cliff continued his botanical explorations leaving much of the work on the farm during his absences to Hilda. In 1968 Cliff and the family moved to Portland to allow Cliff to devote more time to botany.

The 1950s appear to have been particularly busy. Long interested in native bees, Cliff sent his first specimen to the then National Museum of Victoria for identification. Entomologist Tarlton Rayment (1950a) replied: '..... Even your first catch is a New Record for the State.' In response to a request from Tarlton Rayment and detailed instructions on how to proceed, Cliff began studying the life histories and collecting local native bees in 1950. This work led to a very close collaboration between the two. Numerous detailed requests from Rayment followed, some of which required several days of work, or a constant vigil over a long period for a male or a female of a given species. On one occasion Cliff spent two days digging out one *Nomia australica* nest at Bats Ridge, the winding shaft and lateral cells extending almost 1.2 metres to a special moist clay. This work on bees culminated in 1953 with the publication by the Portland Field Naturalists' Club of the book *'Bees of the Portland Region'* by Tarlton Rayment. Altogether Rayment described thirty new species of native bees from Cliff's collections. Of these, three were named after Cliff, four commemorate Portland and two Gorae West. Subsequent to the publication of *'Bees of the Portland Region'*, Cliff collected another twenty species for the Region. Rayment also made a study of the parasitic acarid mites found on the native bees sent by Cliff.

While working on the native bees Cliff extended his studies and started collecting wasps for Rayment. Rayment (1950b) wrote to Cliff: 'Keep your eyes skinned, Cliff, for greenish wasps [*Sericophorus*] about same size as the bees (*Parasphecodes wellingtoni*) in stumps, they make shafts in sandy ground, and I am anxious to get some from your district.' It was only in 1953 that Cliff found the first *Sericophorus* shafts along Cape Nelson Road. He worked on the shafts for fourteen days and subsequently found others at Mt Richmond and at Gorae West. Rayment (1951) wrote: 'Well, Cliff, it looks at first glance that you have been remarkably successful. There were six of the wasps, and one that I

have worked is new – and I think that there will be more.' Cliff collected at least four new species of wasps, one of which was named after him. It is unfortunate that Rayment died before the work on native bees and wasps was concluded as it terminated the prospect of a book on the wasps of the Portland District by the two men. In addition to the bees and wasps, Cliff also collected the type of a new species of blow-fly (*Pollenia tragica* Rayment).

Cliff began a serious study of ants following a meeting early in 1954 with Father John McAreavey, a Master at Xavier College, Melbourne, who was preparing a catalogue of Australian ants (McAreavey, 1954). In July 1954 McAreavey wrote: 'I have just completed naming of your ants, and as you guessed enjoyed it. They are in such good condition and so clearly set out that it is a pleasure and an interest to go through them.' In October 1955 McAreavey wrote: 'You got a nice lot [of ants] –I make it 118 different species representing nearly all the subFamilies.' In addition to new records for Victoria, according to McAreavey, apparently twelve of the ants collected by Cliff represented undescribed species. Unfortunately Cliff had no further contact with McAreavey once the latter left Xavier College. Once again a very fruitful collaboration had terminated prematurely for Cliff.

Cliff's interest in birds was encouraged by people such as Les Chandler and Noel Learmonth. The latter, who published *'Birds of the Portland District'*, indicated that the book 'contains many species that would not have been recorded but for his [Cliff's] field observations, indeed the author would not care to have attempted the book without having the ornithological work of Cliff Beauglehole to draw on.' (Learmonth, 1967). Together with Hilda and other helpers in 1951 he started to collect and record sea birds washed up on the wild storm-lashed 50 kilometre coast of Discovery Bay. The carcasses of birds not collected were deposited behind the first dune to ensure that they were not counted on subsequent beach patrols. Between 1951 and 1963 almost 5000 carcasses were collected by Cliff and his helpers. In one day during August 1959 Cliff and Hilda collected 950 bird carcasses (Corrick, 1971). The flesh was allowed to decompose naturally on wire racks erected on top of the hay shed on the Beauglehole farm. It is said that sometimes it was possible to smell the Beauglehole farm before actually seeing it! Skins and clean skeletons of numerous birds were donated to the National Museum of Victoria and other Australian museums and to Harvard University, the United States of America. This work extended knowledge of the sea birds visiting our shores, and included several new records of species visiting Australia (Corrick, 1971).

In 1957 a group of members of the Victorian Field Naturalists Club of Victoria visited Portland, amongst them the conchologist Charles J Gabriel. Gabriel was hoping to find someone locally to collect tiny land snails for him. Needless to say Cliff took up the challenge. In January 1958 Gabriel wrote: 'Now old chap not in my wildest dreams did I anticipate an answer so quickly to my "S.O.S" for W-Vict Land Shells. It was excellent and please accept my many thanks for your efforts, ….'. Some years later Cliff continued to send molluscs to Fred and Jan Aslin in Mount Gambier.

During the 1950s Cliff, together with Fred Davies of Portland, started a systematic survey of the bone deposits in many of the limestone caves in south-west Victoria. This work arose out of concern at the damage being caused to the caves through destructive activities such as road works and the dumping of rubbish. As in many of his endeavours, Cliff obtained the support of local naturalists and in 1963 they started to excavate McEachern's Death Trap Cave, a chimney cave about 1.2 metres in diameter and 15 metres deep. Several tons of sediments were hauled to the surface and sieved. In this hard physical labour Cliff was assisted by his wife and daughters and collectively they spent hundreds of hours on the project. The identifiable remains of over 2000 animals were recovered and some of the material dated back 5,000 years to the Pleistocene. The highlight of the deposit was the discovery of the remains of a Tasmanian Tiger (Carthew, 1964). In 1964-5 Norman Wakefield became involved in the excavation and assessment

of the material (Wakefield, 1967). Study of this material added greatly to the knowledge of the earlier fauna of the region.

During the 1960s and 1970s Cliff extended his travel visiting Lord Howe Island twice, the Darwin region of the Northern Territory twice, Central Australia seven times and the Kimberley Region of Western Australia four times. In addition to plants, Cliff also collected skinks, geckoes, frogs, lizards, molluscs in Central and Western Australia. However, it was in Victoria that Cliff concentrated his efforts and there is scarcely a road or track in Victoria on which he did not travel at least once.

Cliff received financial support in 1968 from the Royal Botanic Gardens Research Trust Melbourne, a Trust associated with the Maud Gibson Trust, to survey the Grampians. Although the flora of the Grampians was thought to be reasonably well documented, there was a belief that many additional records awaited discovery. This was confirmed by Cliff's studies. The Grampians study was followed by funding from government agencies (mainly the National Parks Service and the Land Conservation Council of Victoria) to subsidise the survey of East Gippsland and other areas of Victoria, and from the Utah Foundation towards a survey of the alpine areas. In his survey work Cliff also received some financial and other support from the Western Victorian Field Naturalists' Clubs Association, from individual clubs, other organisations and individuals. In time the whole of Victoria was surveyed. The results of Cliff's work was published by the Land Conservation Council of Victoria in the form of conservation recommendations. Cliff subsequently published checklists for the entire state in a series of thirteen reports under the title *The distribution and conservation of vascular plants in Victoria.*

Cliff had a very discerning eye especially when it came to detecting differences or unusual forms and missed very little in the field. In a letter supporting Cliff's nomination for the Australian Natural History Medallion, Williams (1967) wrote 'Many of these mosses are almost microscopic in size and indeed I find that I cannot identify many of these without the use of a microscope but Cliff identifies these lowly plants in the field – and he will be 95% right!!' It has not been unusual for many years, and sometimes decades, to elapse before a taxon that Cliff had identified as different was recognised formally and described by a botanist. He seldom resorted to using keys to identification but relied instead on visual recognition and his prodigious memory and ability to recall diagnostic details. In the field when preparing to conduct his surveys, Cliff would plan his collecting sites carefully and ensure that each different community and habitat was visited. On reaching a site, he would walk around and collect and record taxa until he could find no additional taxa before moving on to his next site. During his years of field work he amassed a huge collection of specimens and his collecting numbers exceeded 95,000. Cliff very generously donated the bulk of his private herbarium to the National Herbarium of Victoria many years ago, but he also sent specimens for identification to botanists in a number of other herbaria and his specimens are to be found in each main Australian herbarium and in several overseas. Cliff is the largest single contributor of specimens to the National Herbarium of Victoria and it is unlikely that his record will be bettered. In the case of Victoria, Cliff intended that his specimens would serve as vouchers for his species lists and he attempted to collect each taxon present within the minor 10'×10' grid 'squares' then employed for recording distribution within the state. Many of the specimens were of necessity sterile, but nevertheless they are an important record and often are the only record of the taxon in question from that part of Victoria or indeed from Victoria. Often it is a Beauglehole specimen that alerts one to the existence of an interesting or unusual taxon at a particular site and enables one to re-visit the site. Cliff had tremendous drive, energy and commitment, working from dawn to dusk in the field collecting and recording and half of the night documenting and processing his collections.

Having witnessed the destruction of native habitats in his youth, Cliff was always very environmentally aware and with the passing of the years this awareness intensified. Deeply concerned by the disappearance of natural communities due to agricultural and

urban developments, he was actively involved with a number of other naturalists in many campaigns for environmental conservation. Cliff was one of the foundation members of the Western Victorian Conservation Committee, a sub-committee of the Portland Field Naturalists Club, that was established to spearhead for the fight for the preservation of the Little Desert. The Committee was active in bringing to the attention of successive governments the need to change practices of land management. He was also instrumental in the preservation of the Mount Richmond and the Lower Glenelg National Parks. Although not opposed to the Portland Aluminium smelter, he was opposed to it being sited on the rich wet coastal heathland of Point Danger, arguing that the smelter could readily be moved and built on disturbed pasture land one kilometre inland. Cliff had a long-standing interest in a number of sites in or near Portland such as Fawthrop Lagoon where his contribution is commemorated by a walking track that bears the name 'Cliff's Walkway.' Cliff also led a campaign to prevent McDonald's Family Restaurants from occupying a prime corner site in Portland that resulted in the City Council re-zoning the land and setting it aside permanently as a park. He was concerned not only by the incursion of alien exotics into every plant community in Victoria but also by the spread of native species such as *Acacia longifolia* (Andrews) Willd. subsp. *sophorae* (Labill.) Court and *Pittosporum undulatum* Vent. that were spreading rapidly beyond their former natural ranges and having an adverse environmental effect in every respect as detrimental as that of the worst introduced species. Cliff also lamented the loss of much natural bush to pine and latterly eucalypt plantations.

Always very generous with his time, Cliff was always willing to assist visitors to his area conducting field work, or to give precise directions to one of his collecting localities or provide supplementary information. Whenever a new or challenging piece of field research was required in SW Victoria, Cliff rose to the occasion and responded. Such was his industry, application and enthusiasm, that Cliff invariably surpassed with his contribution the expectations of the person(s) who made the request, more often than not overwhelming them with specimens or records of observations. With an insatiable thirst for knowledge of the natural world, Cliff was more or less the complete naturalist, a rarity in this day of increasing specialisation. What makes Cliff's contributions prior to 1968 when the family moved to Portland more remarkable is that in addition to devoting so much energy to his natural history interests, he also had to earn a living operating his farm although in this latter enterprise Hilda played a crucial role.

Cliff always relied initially on specialists to identify his collections. In the case of the specialist entomologists with whom he collaborated, each predeceased Cliff by many years and consequently the collaborative projects lapsed prematurely which would have been a great disappointment for Cliff. Unfortunately the government herbaria that Cliff turned to for assistance were basically unable to cope with the quantity of specimens and meet his deadlines which were often incompatible with institutional priorities. I recall vividly receiving several consignments of specimens from Cliff. Unlike most other collectors who sent in an occasional box of specimens, Cliff would send in cubic metres of specimens.

Cliff was a foundation member of the Portland Field Naturalists Club and of the Western Victorian Field Naturalists Clubs Association. Always an active member of the Portland Field Naturalist Club, he encouraged many country naturalists to develop an interest in their environment and to monitor and document the vegetation. Cliff became a very important figure and an inspiration to many of these naturalists who sent specimens to him for identification or sought his advice. Cliff received Life Membership of the Portland Field Naturalist Club in 1962 in recognition of his contributions. In 1982 he was elected an Honorary member of the Field Naturalists Club of Victoria in recognition of 40 years of continuous membership. Always very active, he was a member of many other Clubs, Associations and Societies.

In 1971 Cliff was awarded the Australian Natural History Medallion for services to

natural history. In 1984 he was awarded the 'Medal of the Order of Australia' (OAM) for his services to botany, conservation and ornithology.

Much of what Cliff accomplished would not have been possible without the support of Hilda. It was very fitting that The Portland Field Naturalists Club recognized her contribution and presented her with a Life Membership certificate on 10 October 1962 with the following words inscribed; 'presented to Mrs. Hilda Beauglehole who in so large a measure has assisted her husband in the splendid work he has achieved.'

Cliff's legacy will live on through his collections which constitute a huge resource from which future generations will continue to derive benefits, his publications, and from his vision in campaigning actively for several natural areas to be set aside as national parks.

Cliff is survived by his wife, two daughters, seven grandchildren and two great-grandchildren.

Taxa named after Cliff:

Algae	***Helminthocladia beaugleholei*** Womersley
	Nitella tasmanica Muell. ex A.Braun subsp. ***gelatinifera*** R.D.Wood var. ***beaugleholei*** R.D.Wood
Moss	***Phascum beaugleholei*** I.G.Stone
Vascular plants	*Bassia beaugleholei* Ising (now a synonym of ***Sclerolaena diacantha*** (Nees) Benth.)
	Caladenia beaugleholei D.L.Jones (now a synonym of ***Arachnorchis flavovirens*** (G.W.Carr) D.L.Jones & M.A.Clem.)
	Epilobium brunnescens (Cockayne) P.H.Raven & Engelhorn subsp. ***beaugleholei*** K.R.West & P.H.Raven
	Lobelia beaugleholei Albr.
	Prasophyllum beaugleholei Nicholls (now a synonym of ***Corunastylis nuda*** (Hook.f.) D.L.Jones & M.A.Clem.)
	Solanum beaugleholei D.E.Symon
	Stylidium beaugleholei J.H.Willis
	Utricularia beaugleholei R.J.Gassin
	Villarsia umbricola Aston var. ***beaugleholei*** Aston
Lichen	***Heterodea beaugleholei*** Filson
Bees	***Exoneura cliffordiella*** Rayment
	Hylaeus cliffordiellus Rayment
	Megachila cliffordii Rayment
Wasp	***Serocophorus cliffordii*** Rayment

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In addition, Cliff contributed about 100 articles that were published under the title 'Nature Notes' in the Portland Observer edited by B.E. Carthew during the years 1941–1949, and numerous articles to the Australian Conservation Foundation Portland Chapter Newsletter and to the Western Victorian Conservation Committee Newsletter.

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I am most grateful to Hilda Beauglehole and Valerie and Yvonne for sharing with me memories of some of the early days with Cliff and for providing the photograph reproduced above, and to Margaret Corrick for sharing recollections her association with the Beauglehole family when she lived in southwestern Victoria.

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Muelleria 16: 81–82 (2002)

Some new combinations and a new hybrid genus in Orchidaceae: *Diurideae*, for eastern Australia

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Abstract

New combinations and a new hybrid genus are created for the Orchidaceae tribe *Diurideae* in eastern Australia. The following new combinations are made in *Arachnorchis* D.L.Jones & M.A.Clem. and *Simpliglottis* Szlachetko — *Arachnorchis* ×*variabilis* (Nicholls) Jeanes, *Simpliglottis grammata* (G.W.Carr) Jeanes, *Simpliglottis jeanesii* (D.L.Jones) Jeanes and *Simpliglottis triceratops* (D.L.Jones) Jeanes. The following new hybrid genus is created followed by a new combination within that genus — ×*Chilosimpliglottis* Jeanes, ×*Chilosimpliglottis pescottiana* (R.S.Rogers) Jeanes.

Introduction

The past couple of years have seen a flurry of activity in the reclassification of parts of the primarily Australian orchid tribe *Diurideae* (Hopper & Brown 2000, 2001a, 2001b; Jones *et al.* 2001; Szlachetko 2001a, 2001b; Jones *et al.* 2002). These various works have given rise to conflicting classifications at the generic and subgeneric levels as well as the publication of many invalid names. Furthermore, the authors have overlooked several taxa and hence some of the necessary combinations have not been made into these new taxonomic systems. The opportunity is here taken to create the necessary new combinations at the generic level for those taxa occurring in eastern Australia. The creation of these new combinations will benefit flora writers, compilers of flora lists and land management authorities who often work within a legislative framework that demands validly published binomials for the taxa with which they deal.

Taxonomy

Arachnorchis* x*variabilis (Nicholls) Jeanes, *comb. nov.*
Basionym: *Caladenia variabilis* Nicholls, *Victorian Naturalist* 66: 223, figs L & M (1950).
An apparent natural hybrid between *Arachnorchis orientalis* (G.W.Carr) D.L.Jones & M.A.Clem. and *Arachnorchis tessellata* (Fitzg.) D.L.Jones & M.A.Clem. from south-eastern Victoria. Natural hybrids of similar appearance can be derived from hybridization between other taxa (Jeanes & Backhouse 2001).

x*Chilosimpliglottis* Jeanes, *hybrid gen. nov.*
This hybrid genus is the result of natural hybridization between the genera *Chiloglottis* R.Br. and *Simpliglottis* Szlachetko. One named taxon is currently recognised.

x*Chilosimpliglottis pescottiana* (R.S.Rogers) Jeanes, *comb. nov.*
Basionym: *Chiloglottis pescottiana* R.S.Rogers, *Proc. Roy. Soc. Victoria* new ser. 30: 139, t.25 (1918).
This natural hybrid between *Chiloglottis trapeziformis* Fitzg. and *Simpliglottis valida* (D.L.Jones) Szlachetko occurs in New South Wales and Victoria, and has been observed at a number of sites where the ranges of the parent species overlap.

Simpliglottis grammata (G.W.Carr) Jeanes, *comb. nov.*
Basionym: *Chiloglottis grammata* G.W.Carr, *Indigenous Flora & Fauna Association Miscellaneous Paper* 1: 20 (1991).

Simpliglottis jeanesii (D.L.Jones) Jeanes, *comb. nov.*
Basionym: *Chiloglottis jeanesii* D.L.Jones, *Orchadian* 12(5): 233 (1997).

Simpliglottis triceratops (D.L.Jones) Jeanes, *comb. nov.*
Basionym: *Chiloglottis triceratops* D.L.Jones, Contributions to Tasmanian Orchidology-3: A Taxonomic Review of *Chiloglottis* R.Br. in Tasmania, *Australian Orchid Research* 3: 66 (1998).

Note: *Simpliglottis* Szlachetko differs from *Chiloglottis* in a number of important morphological characters. In *Simpliglottis* the leaves are generally broader and lack undulate margins, the scape is generally shorter (although it does elongate after anthesis) and stouter, the flower is usually larger, the petals are spreading or incurved (deflexed against the ovary in *Chiloglottis*), the labellum is extremely mobile (more or less fixed in *Chiloglottis*), elliptic, ovate or cordate in shape (rhomboid or trapezoid in *Chiloglottis*) and the lamina calli are generally fewer, less crowded and of fairly uniform appearance.

The results of recent molecular studies conducted on the group by Jones *et al.* (2002) demonstrate monophyly for *Simpliglottis* within *Chiloglottis sens. lat.* although they have chosen to recognise *Simpliglottis* at the subgeneric level only.

Acknowledgments

My thanks go to David Jones (CANB), Jim Ross (MEL) and Neville Walsh (MEL) for kindly checking an earlier draft of this paper.

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Muelleria 16: 83–86 (2002)

Nymphoides simulans (Menyanthaceae): a new species from northern Australia

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Abstract

Nymphoides simulans Aston *sp. nov.*, a white-flowered species from northern Queensland and north-eastern Northern Territory, is described and its diagnostic features are discussed. The species is almost identical with *N. spongiosa* Aston in leaves and seeds, but differs from that species in several floral features.

Introduction

This paper is the sixth in a series connected to a revision of *Nymphoides* Séguier in Australia. Ten new species have been published in prior papers (Aston 1982, 1984, 1986, 1987, 1997) but material of the species now being described has been set aside for some time awaiting fuller investigation. Recent examination of seeds by Scanning Electron Microscope (Aston, in preparation) has assisted in clarifying the taxon's status. *Nymphoides simulans* Aston belongs in the informal 'indica group' defined in Aston (1982, p. 35).

Taxonomy

Nymphoides simulans Aston, *sp. nov.*

Nymphoides spongiosa Aston simulans arcte habitu, foliis, fructis et seminibus, sed floribus 4-partitis (interdum 3-partitis vel 5-partitis) homostylis, antheris stigmata aequantibus praecipue differt.

Type: Queensland, Cape York Peninsula, 3.2 km E of "Musgrave" along the "Musgrave" to "Marina Plains" road, 13 May 1982, *H.I. Aston 2255* (holotype MEL *612170*; isotypes BRI, MEL *612171*, MEL spirit).

Apparently annual. *Petiole-like stems* few to many (>20), arising from the plant base, slender, flexuose, 3.5-42 cm long, <1mm diam. *True petiole* apparently absent. *Leaf blades* floating, elliptic to elliptic-oblong to broad-ovate in outline, deeply cordate (the lobes c. (25%-)40%-50%(-55%) of the total blade length and separated by a sinus of (20°-)30°- 75°(-100°) angle), obtuse, entire, (1.0-)2.0-3.5(-4.2) cm long, (0.7-)1.5-2.5(-3.5) cm wide, deep green or maroon-purple and shining above, white-translucent and spongy beneath; spongy tissue smooth-surfaced, not rugose. *Juvenile leaves* basal, submerged, very thin-textured, light green and almost translucent, not spongy; blades variable in shape, narrow-elliptic, oblanceolate to obovate, or broad-ovate to deltoid, c. 7-22 mm long, tapered into dorso-ventrally flattened petioles c. 5-35 mm long. *Inflorescence* as for the 'indica group'; pedicels subtended by ± broad-ovate, membranous, translucent bracts c. 2-3.5 mm long. *Pedicels* 5-11(-20), emerging erect through the sinus when in flower, very slender, 4-15(-20) mm long, < 0.5 mm diam. *Flowers* (3)4(5)-partite, homostylous. *Calyx lobes* lanceolate, acute, membranous, mostly purplish-translucent, often slightly outcurved at the apex when in fruit, 1.6-2.7 mm long. *Corolla* (4-)5-7 mm span, white with a yellow throat. *Corolla lobes* narrowly oblanceolate (almost linear) to broadly oblanceolate; mid-section glabrous except for c. 8-20 fine papillae spaced in a transverse band just above its base, the band sometimes discontinuous, the papillae then only at the sides or at the sides and centre of the band position; central longitudinal keel

or wing on mid-section absent; side-wings extending from the apex of the lobe to half or two-thirds of the lobe length, varying from near-entire with 1-few crenulations or short laciniae at the apex, to strongly crenulate or with several deep-cut laciniae spaced along the whole margin. *Corolla tube* papillae short, each several cells long, clustered c. 8-12 together at the summit of a short common stalk, or the cluster sometimes sessile. *Stamens* with straight filaments 0.50-0.85 mm long; anthers versatile, ± broad-obloid with the

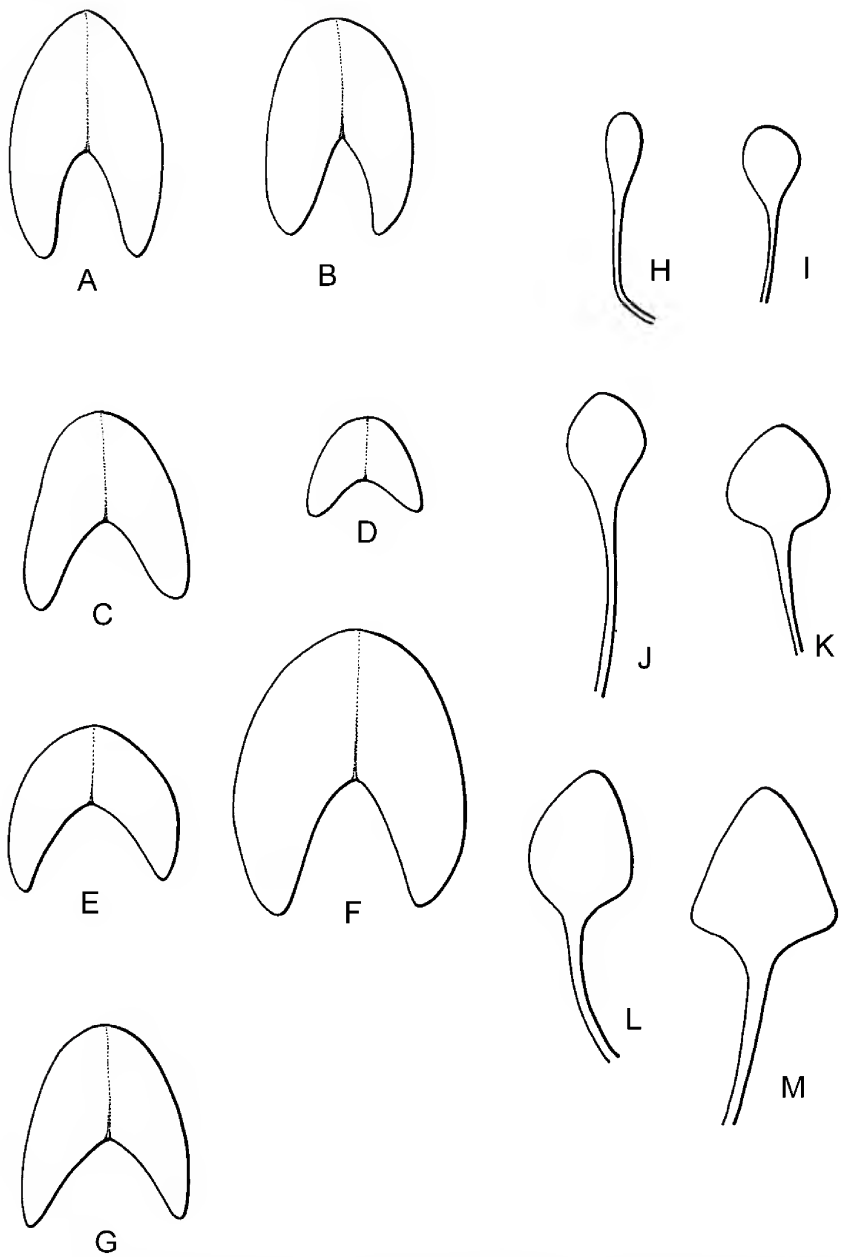

Figure 1. *Nymphoides simulans*. **A-G** Outline of floating blades of mature leaves, × 1: **A** (*Craven 3203*), **B-D** (*Aston 2251*), **E-F** (*Aston 2247*), **G** (*Aston 2255*). **H-M** Outline of juvenile leaves, x 1: **H-K** (*Aston 2255*), **L-M** (*Craven 3203*).

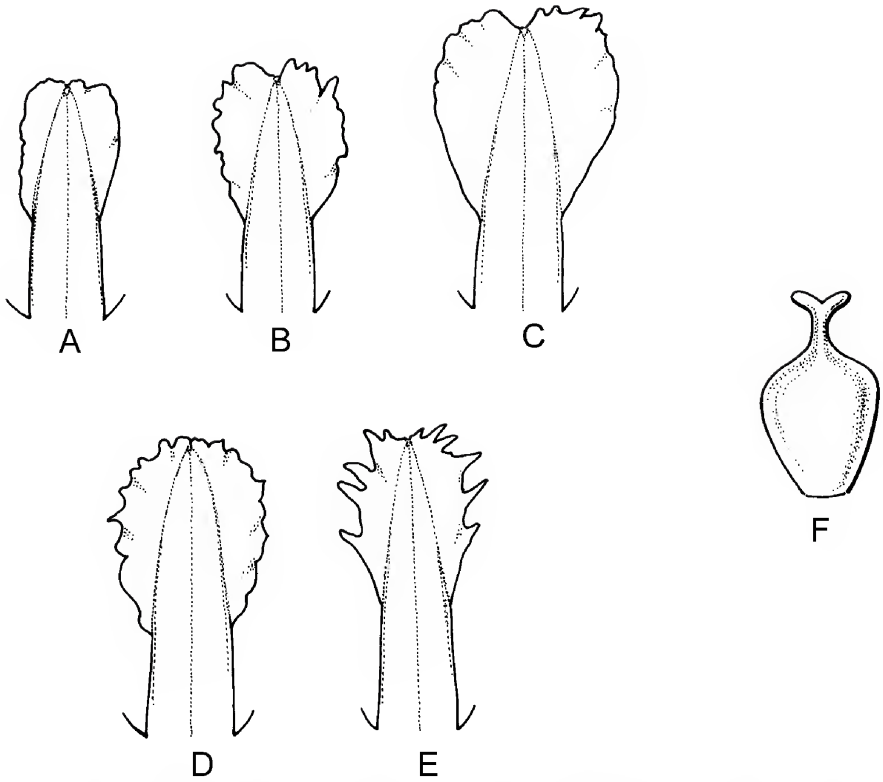

Figure 2. *Nymphoides simulans*. **A-E** Outline of corolla lobes to show the crenulate to laciniate wing margins, × 15: **A-B** (*Aston 2247*), **C-E** (*Aston 2255*). **F** Outline of gynoecium showing ovary shape and gentle contraction into the style, × 20 (*Aston 2255*).

length slightly > to slightly < width, (0.25-)0.30-0.45 mm long, (0.225-)0.25-0.40 mm wide. *Gynoecium* c. (1.20-)1.3-1.5(-1.7) mm long; ovary obovoid to broadly obovoid, the shoulders at the summit contracted gently rather than abruptly into the style; placentas 2, short, about one-quarter to one-third of the capsule length, positioned centrally down the ovary wall; ovules c. 5-19; style (0.15-)0.20-0.25(-0.30) mm long; stigmas 2, each a broad, papillate, slightly lobed wing c. (0.20-)0.25(-0.35) mm long. *Capsule* broadly obovoid or sometimes broadly obloid, from a little less than to equal to or rarely greater than the calyx, 2.0-2.8 mm long, 1.4-2.05 mm wide. *Seeds* (4-)7-19 per capsule; body of seed near-globose but slightly to moderately laterally compressed, 0.575-0.85 mm long, 0.55-0.775 mm wide, 0.35-0.60 mm thick, cream-straw to light brown-grey or brown-black when mature, usually densely covered with very short, usually broadly conical but obtuse, occasionally convex, tubercles which arise one from each cell and give the seed surface a densely granular appearance; seed faces sometimes smooth, the tubercles present only on or close to the seed edges and gradually diminishing in length from the edge towards the face; basal caruncle present, circular, pale, typically thick and conspicuous, sometimes thin. (Figs 1& 2).

Phenology: Flowers and fruits recorded April to June.

Etymology: The specific epithet refers to the similarity of *N. simulans* to *N. spongiosa* except in floral characters.

Distribution and Conservation Status: Known from the Northern Territory and Queensland, north of 18°S latitude and east of 132°E longitude. Apparently most prolific

in Queensland, on Cape York Peninsula, particularly in the "Musgrave" to Hann River region but extending south to about Mt Molloy. There are only four widespread records from the remainder of the known range, namely two from the East and South Alligator River regions of the Northern Territory, and two from the southern edge of the Gulf of Carpentaria (Bing Bong, N.T. and near Westmoreland, Qld). *Nymphoides simulans* is probably well under-collected across its wide geographical range and is apparently under no particular conservation threat at this time.

Habitat: Occurs in still, sometimes gently flowing, temporary fresh waters to 50 cm deep in seasonally flooded swamps, lagoons, or roadside ditches, persisting for a while on saturated marginal zones as waters recede. Substrate usually sand or sandy gravel, sometimes mud. Recorded "in a sedge swamp through a *Melaleuca* flat" (*Aston 2259*) and in "*Melaleuca viridiflora* closed woodland on a depression" (*A.V. Slee* et al *2880*). In two populations (*Aston 2247* and *Aston 2255*) *N. simulans* occurred intermingled with *N. triangularis* Aston and in another population (*Aston 2251*) it was intermingled with *N. parvifolia* (Griseb.) Kuntze.

Notes: *Nymphoides simulans* resembles *N. spongiosa* (Aston 1982, pp. 45-48, fig. 4) very closely in habit, leaf, fruit and seed, but differs chiefly in having 4-partite flowers (occasionally 3- or 5-partite), and in being homostylous with anthers and stigmas held at the same level at anthesis. There are also other floral differences. Flowers which I have seen either in the field, or in spirit, or softened from dried collections, are smaller (corolla span c. 5-7 mm) than those from *N. spongiosa* (10-20 mm, occasionally as short as 7 mm), and have the wings of the corolla lobes usually shorter in relation to the lobe length, narrower, and crenulate to laciniate rather than entire. Anthers of *N. simulans* are smaller (0.25-0.45 mm long) and squatter, sometimes even wider than long, whereas those of *N. spongiosa* are about 0.5-0.7 mm long and 1.5 times as long as broad. The ovary of *N. simulans* also differs, that of *N. spongiosa* being more globular and abruptly contracted into the style.

Representative Specimens Examined (17 collections examined): **Queensland**: Cape York Peninsula, 5.3 km NW of the Hann River crossing of the Laura to Coen road, 11 May 1982, *H.I.Aston 2247* (MEL); 11.6 km SE of the Morehead River crossing of the Laura to Coen road, 12 May 1982, *H.I.Aston 2251* (BRI, MEL); 5.4 km S of Musgrave along the Laura to Coen road, 14 May 1982, *H.I.Aston 2264* (BRI, MEL, NSW); About 140 km S of Cooktown on the Cairns road, 9 April 1975, *L.A.Craven 3203* (BRI, CANB, MEL); "Westmoreland" homestead lagoon, 11 May 1974, *S.Jacobs 1547* (MEL, NSW). **Northern Territory**: Bing Bong Station, 8 June 1971, *C.R.Dunlop 2250* (DNA); Grown [at CSIRO Berrimah] from soil collected on East Alligator floodplain, 12°14' S, 133°01' E, 29 April 1994, *S.Seddifield s.n.* (DNA).

Acknowledgements

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Variation within *Asterolasia asteriscophora sensu lato* (Rutaceae: Boronieae) and the recognition of new taxa in eastern Australia

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Abstract

Asterolasia asteriscophora (F.Muell.) Druce *sensu lato* exhibits considerable variation in a range of floral and vegetative characters. Phenetic analysis of 14 morphological characters for 17 populations of *A. asteriscophora sensu lato* has elucidated three new taxa: *A. asteriscophora* subsp. *albiflora* B.J.Mole, *A. rupestris* B.J.Mole and *A. rupestris* subsp. *recurva* B.J.Mole, and supports the recognition of *A. buckinghamii* (Blakely) Blakely and *A. buxifolia* Benth. as distinct species. Isozyme electrophoresis and subsequent interpretation proved problematic, however allele frequency data for a limited number of enzyme systems were broadly congruent with morphometric analyses. A key and detailed taxonomic descriptions are provided.

Introduction

Asterolasia asteriscophora (F.Muell.) Druce is a variable taxon of eastern Australia currently suspected to contain several infraspecific taxa (Wilson 1970, 1980, *ined.*; Duretto 1999). The species was first described by Ferdinand von Mueller (1855) as *Phebalium asteriscophorum* F.Muell., from material he collected in 1852 at Mt Disappointment, north of Melbourne, Victoria. Bentham (1863) placed it in *Asterolasia* F.Muell. as *A. muelleri* Benth. Druce (1917) noted that Bentham had not used the correct epithet when transferring the species to *Asterolasia* and so made the correct combination.

As currently circumscribed (see Porteners 1991; Duretto 1999), *A. asteriscophora* is a slender, erect, many-branched shrub, 1–2 m in height. Leaves, stems and the abaxial surface of the petals are covered with an indumentum of stalked, multiangular, stellate trichomes. The leaves are highly variable in shape and size, spathulate to obovate, oblong-cuneate or elliptic, 3–34 mm long, and 2–12 mm wide. A form with obcordate leaves is known from Mt Kaputar National Park in New South Wales. The inflorescences are terminal or axillary, solitary or in few flowered umbels, with the terminal flower reaching anthesis first, followed by the lateral flowers. Petals are usually yellow though plants from the Emerald area (Vic.) have white petals. The range of the species' extends from the Macedon Ranges in Victoria to the Torrington district in northern New South Wales (Fig. 1). Although the species is widespread, populations are generally small and disjunct.

The form of *A. asteriscophora* with white petals noted by Wilson (*ined.*) and Duretto (1999) from Emerald, Victoria, has been described as *Eriostemon spathulifolius* Gand. (Gandoger 1913), but has not been formally recognised in recent flora treatments (Willis 1973; Duretto 1999). This form is located within a residential zone and is now only known from three localities in the Emerald-Avonsleigh district, c. 45km ESE of Melbourne, Victoria. Formal taxonomic recognition of this form would have immediate conservation implications as the populations are threatened directly and indirectly by residential development.

Wilson (1998) noted that in the *Flora of New South Wales* (Porteners 1991) *A. buxifolia* Benth. was not accounted for. This is also the case for *A. buckinghamii* (Blakely) Blakely, although this was not noted by Wilson (1998). Wilson (1970), however, listed *A. buckinghamii* as an accepted taxon. In order to resolve taxonomic problems in the *A. asteriscophora* species group, specimens were subjected to a phenetic analysis. It was

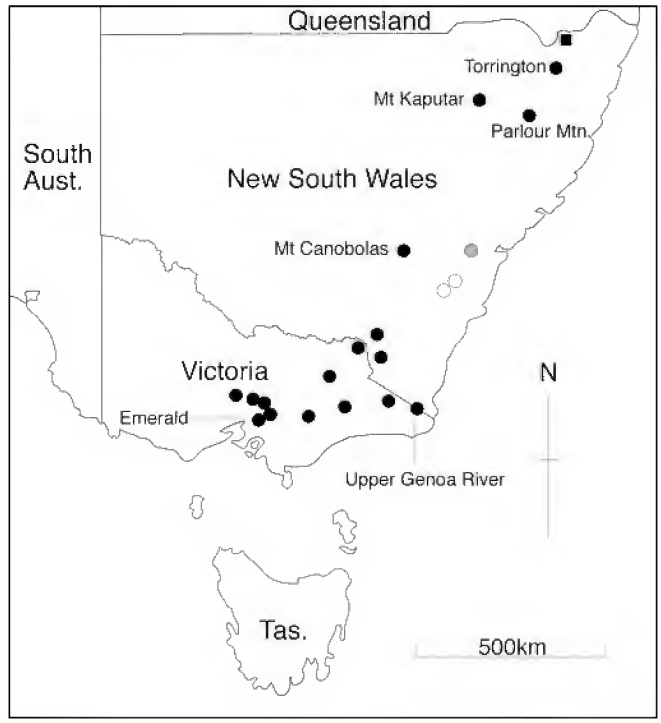

Figure 1. Known distribution of *Asterolasia asteriscophora sensu lato* (black circles), *A. buxifolia* (shaded circle), and *A buckinghamii* (open circles). All populations were sampled in this study except for Mt Canobolas, Upper Genoa River, and *A. buxifolia*, which are represented by herbarium specimens. The black square represents the single population of *A. correifolia* sampled for isozyme analysis. Under the new classification proposed here, populations from Mt Kaputar and Mt Canobalas are *A. rupestris* subsp. *rupestris*; those from Parlour mountain are *A. rupestris* subsp. *recurva*; those from Emerald are *A. asteriscophora* subsp *albiflora*; other populations from Victoria and NSW are *A. asteriscophora* subsp *asteriscophora.*

evident prior to analyses that *A. buxifolia* was distinct based on its glabrous ovaries, (character 13), however, as this taxon had apparently been treated as conspecific with *A. asteriscophora*, it was included in analysis 1.

Methods and Materials

POPULATION SAMPLING

Seventeen populations representing the known geographic range of *A. asteriscophora sensu lato* (Fig. 1) were sampled between November 1998 and January 1999. For the morphometric analysis, five individuals were sampled from each population. The exception was the population at Pine Mountain which consisted of only six individuals, from which only a small sample from one individual was taken. Herbarium specimens from BRI, CANB, MEL, MELU, NE, NSW, and K were used to supplement field collections for areas not sampled during field work. Herbarium acronyms follow Holmgren *et al.* (1990).

A subset of 12 populations was sampled for isozyme analysis. For each population, 3–12 leaves were collected from 15 individuals. Fifteen individuals of a closely related but morphologically distinct species, *A. correifolia* (A.Juss.) Benth., were also sampled for comparison. Three voucher specimens were collected for each population.

MORPHOLOGICAL CHARACTERS

One hundred and sixteen specimens (Appendix 1) were scored for 14 morphological characters (Table 1) reflecting the variation in leaf shape, leaf size, density and type of indumentum, petiole length, petal colour, peduncle and pedicel length, and number of flowers per inflorescence. All continuous characters were scored as an average of measurements from five organs (where five organs were available). Most characters are self explanatory, but a few require further clarification.

The prophylls of *A. asteriscophora sensu lato* are leaf-like and may be confused with leaves. They can be distinguished on the basis of their position. Prophylls subtend the pedicels, and are similar in shape and size to one another but consistently different from leaves subtending an inflorescence or axillary shoot. Leaves subtending an inflorescence or axillary shoot were interpreted as true leaves.

Leaf size is highly variable within populations and appears to be related to the age of the plant. Relatively young, vigorous plants tend to have larger leaves than more mature plants. Only mature specimens were used in this analysis.

Ratio characters, leaf length:leaf width (character 2), leaf length:distance to widest point from leaf base (character 3), and petal length:petal width (character 10), were used to quantify the shape of leaves and petals respectively. Since the shape of leaves and petals was consistent on any given individual, length and width measurements were considered to be measuring the same character (leaf size). Therefore width measurements for both leaves and petals were removed prior to analysis.

Table 1. Characters scored for morphometric analyses.

Characters were used in both analyses unless indicated

Continuous characters

1. Leaf length (along midvein) (mm)
2. Leaf length : leaf width
3. Leaf length : distance to widest point from leaf base (mm)
4. Length of petiole (mm)
5. Trichome density (per mm square) on adaxial leaf surface
6. Average number of flowers per unit inflorescence
7. Length of peduncle at anthesis (mm)
8. Length of pedicel at anthesis (mm)
9. Length of petal (mm)
10. Petal length : petal width

Binary characters

11. Leaf margins recurved/not recurved 0/1
12. Indumentum on abaxial petal surface multiangular/globular stellate 0/1 (analysis 1 only)
13. Ovary glabrous/stellate indumentum 0/1 (analysis 1 only)

Multistate characters

14. Petal colour white(0)/pale lemon(1)/yellow (2)

Trichome density of the adaxial surfaces of leaves (character 5) was calculated using transparent grid paper (1 mm^2). The grid was placed over a leaf surface midway along the leaf, avoiding the midrib, and the number of trichomes in five separate grids were counted, and the average recorded for each leaf. The number recorded for each specimen is the average of five leaves.

Peduncle length (character 7) and pedicel length (character 8) showed considerable variation within a single individual but meaningful comparisons could be made by measuring only those subtending flowers at anthesis. Generally, pedicel length was greater at anthesis than in bud and greater still in fruit. Some specimens had solitary flowers while others had umbels of three or more flowers. When specimens had umbels of three or more flowers, the pedicel of the central flower was measured for consistency.

SCANNING ELECTRON MICROSCOPY

Adaxial and abaxial leaf surfaces of specimens were examined to ascertain the structure of trichomes present, and to illustrate variation in trichome density. Leaves stored in 70 % ethanol were air dried and mounted on stubs with the aid of carbon adhesive discs and conductive silver paint. Samples were coated with gold using an Edwards Sputter Coater S150 B and examined and photographed using a Phillips XL 30 Field Emission Scanning Electron Microscope.

MULTIVARIATE PATTERN ANALYSIS

Multivariate pattern analyses were completed using the computer package PATN (Belbin 1987). A distance matrix was constructed using the Manhattan metric distance measure. The Manhattan metric depends on the range of all characters used for its scaling factor (Sneath and Sokal 1973), therefore, all data were range standardised prior to each analysis to ensure all characters were of equal weight (Sneath & Sokal 1973; Milligan & Cooper 1988). Two sets of analyses were performed, the first included all specimens, while the second excluded specimens of *A. buxifolia*. Both sets of analyses included an Unweighted Pair Group Method of Averaging (UPGMA) hierarchical classification to identify discrete clusters of individuals and a Non Metric Multidimensional Scaling (NMDS) ordination to identify continuous variation and clusters of individuals.

Co-phenetic correlation coefficients for dendrograms were calculated using a Spearman Rank correlation coefficient in the computer program SPSS (Norusis 1990). Cramer values, which give a measure of the discriminating powers of characters for each group identified in a classification (Belbin 1987), were also calculated. Cramer values have a scale of 0–1, with a higher value indicating a higher discriminating power for the relevant character.

Twenty different starting configurations were used in each NMDS analysis to ensure that the ordination which best fits the data was obtained. This is identified by the run with the lowest stress value, which is an indication of the degree of correlation of the distances between individuals in the ordination and distances in the original distance matrix. There was little difference in stress values for all twenty runs, indicating global minima were obtained (Kruskal & Wish 1973). Three-dimensional and two-dimensional analyses were performed. Because three dimensional ordinations provided only marginally greater information on groups than two-dimensional ordinations, only two-dimensional ordinations are presented here.

A Minimum Spanning Tree (MST) was also calculated. A MST connects all individuals with single linkages so that the shortest possible tree is constructed with no closed loops. It gives a more accurate portrayal of inter-entity distance than an ordination, which loses some of this information, particularly when the number of dimensions used is low (Belbin 1987).

ISOZYMES

One hundred and ninety five individuals from 13 populations (15 individuals per population) were assayed for isozyme polymorphisms. Small pieces of leaf were ground in the grinding buffer of Warburton *et al.* (2000) in Eppendorf tubes using a hand held electric drill.

Protein electrophoresis was carried out using the Titan III cellulose acetate system (Helena Laboratories) as described by Coates (1988). Thirteen different enzyme systems often found to be polymorphic in plants were tested using three continuous running

buffers: (1) TEM, (2) CM (Warburton *et al.* 2000) and TG (Hebert & Beaton 1989). Only four enzymes run in TG running buffer gave reliable banding patterns for the majority of samples: aspartate aminotransferase (AAT, EC 2.6.1.1), fumarate (FUM, EC 4.2.1.2), glucose-6-phosphate isomerase (GPI, EC 5.3.1.9) and phosphoglucomutase (PGM, EC 5.4.2.2). After electrophoresis for 20–30 min, depending on the enzyme, gels were stained using an agar overlay of 6 ml of 1% agar at 45°C added to the appropriate stains, described by Hebert and Beaton (1989) but scaled down to a volume of 1.2 ml.

Each zone of activity on gels was assumed to represent a single locus. Alleles were numbered according to their electrophoretic mobility and recorded in a table for further analysis.

ANALYSIS OF ISOZYME DATA

The BIOSYS-1 program of Swofford and Selander (1981) was used to analyse allele frequency data. Phenetic analysis was carried out using Nei's genetic distance (Nei 1978) and the UPGMA hierarchical clustering technique of Sneath and Sokal (1973). Phylogenetic analysis was carried out using the modified Rogers' Distance (Wright 1978) and the Distance Wagner method. Both methods gave similar results, and only the Distance Wagner method is presented here.

TAXON DESCRIPTIONS

Descriptive terminology follows Weston (1990) for inflorescence structure, though we use pedicel instead of anthopodium, and Hewson (1988) and Theobald *et al.* (1979) for trichomes. Conservation codes follow Briggs and Leigh (1996).

CULTIVATION

Plants from Emerald and Torrington, with extremes in trichome density (measured on the adaxial leaf surface), were propagated asexually. Cuttings were prepared from firm young growth and treated with Indole Butyric Acid 3000 ppm. Cuttings formed adventitious roots in 4–6 weeks. These were grown under similar conditions in a glasshouse to determine if trichome density reflects genetic differences or is a response to environmental conditions.

Results and Discussion

MORPHOMETRIC ANALYSIS

Analysis 1 (specimens 1–116, characters 1–14): Analysis 1 included 116 specimens of *A. asteriscophora, A. buckinghamii* and *A. buxifolia*. Both the UPGMA classification (Fig. 2) and the ordination (Fig. 3) indicate two major groups (groups A and B). The high co-phenetic correlation coefficient for the classification (0.722) and the low stress value for the ordination (0.182) indicate a minimal and acceptable loss of information from the original distance matrix. Group A incorporates two type specimens (specimen 116 - lectotype) and (specimen 115 - residual syntype) of *A. buxifolia* from the Blue Mountains, New South Wales. They are distinct from all other specimens in having a glabrous ovary (character 13), a glabrous adaxial leaf surface (character 5), and globular stellate trichomes on the abaxial surface of petals (character 12).

Group B (specimens 1–114) consists of specimens from New South Wales and Victoria characterised by a stellate indumentum on the ovary (character 13) and adaxial surface of the leaf (character 5), and stalked, multiangular stellate trichomes on the abaxial surface of petals (character 12). The classification indicates that *A. asteriscophora sensu lato* contains a number of subgroups. While the ordination indicates *A. buckinghamii* is a distinct group, other subgroups identified in the classification are not as clearly separated due to the compression of ordination space by the inclusion of *A. buxifolia*. Therefore the data were re-analysed omitting *A. buxifolia.*

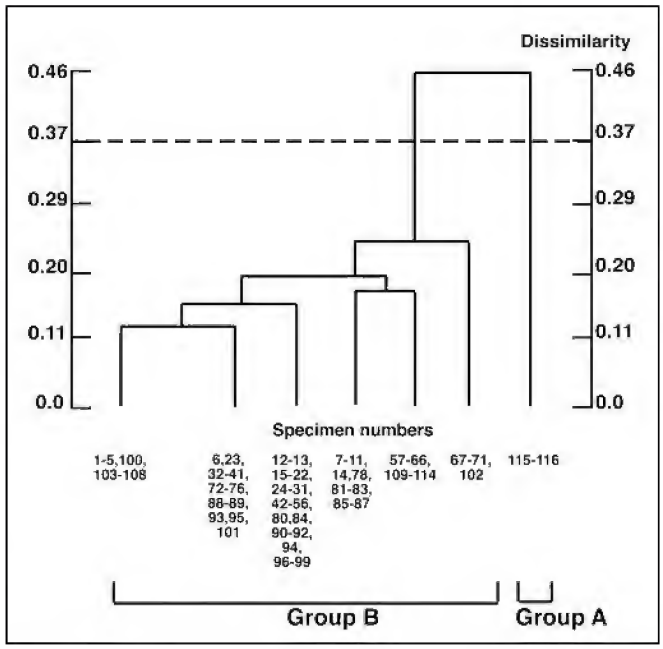

Figure 2. UPGMA classification, analysis 1, specimens 1–116, truncated as indicated by the dashed line. Group A: *Asterolasia buxifolia*. Group B: *A. buckinghamii*, and *A. asteriscophora*. Co-phenetic correlation coefficient = 0.722.

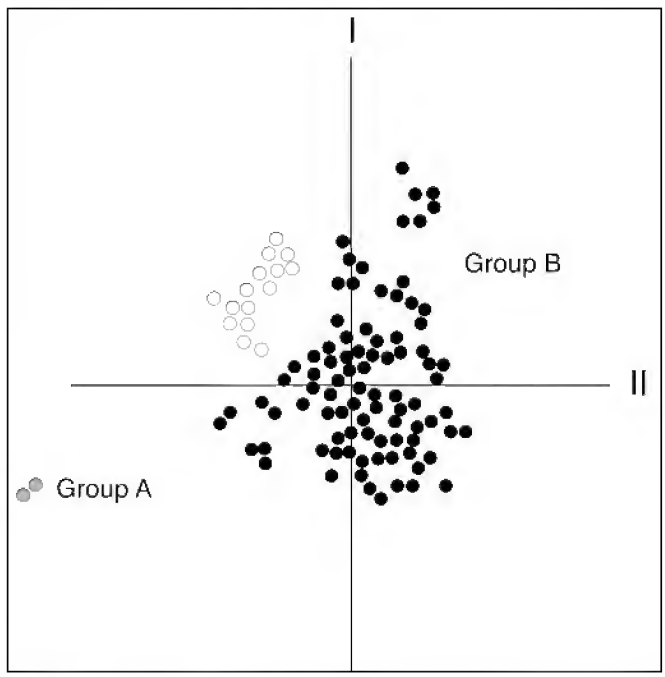

Figure 3. NMDS ordination, vector I versus vector II, analysis 1, specimens 1–116. Symbols are: *Asterolasia buxifolia*, Group A (shaded circles); *A. buckinghamii*, Group B (open circles); *A. asteriscophora sensu lato* Group B (solid circles). Stress = 0.182.

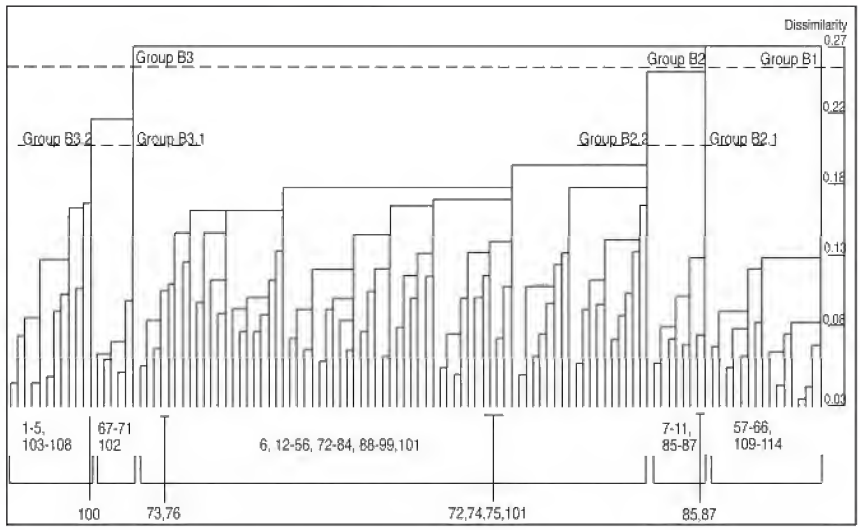

Figure 4. UPGMA classification, analysis 2, specimens 1–114. Co-phenetic correlation coefficient = 0.707. Numbered specimens are referred to in text.

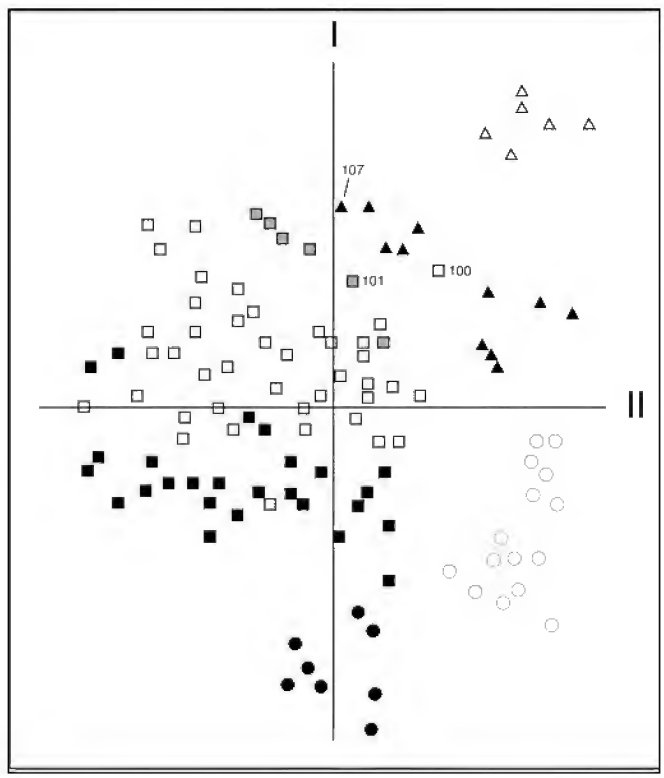

Figure 5. NMDS ordination, vector I verses II, analysis 2, specimens 1–114. Symbols are: *Asterolasia buckinghamii*, Group B1 (open circles); Group B2.1 (solid circles); Group B2.2 Melbourne region (solid squares); Group B2.2 East Gippsland and southern NSW (open squares); Group B2.2 Torrington (shaded squares); Group B3.1 (open triangles); and Group B.3.2 (solid triangles). Numbered specimens referred to in text. Stress = 0.182.

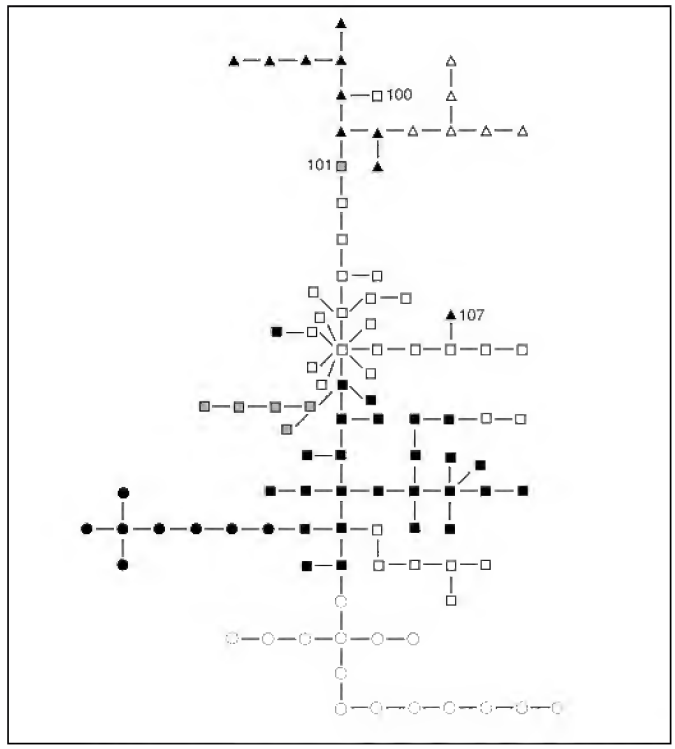

Figure 6. Minimum spanning tree, Analysis 2, specimens 1–114. Symbols are: *Asterolasia buckinghamii*, Group B1 (open circles); Group B2.1 Emerald (solid circles); Group B2.2 Melbourne region (solid squares); East Gippsland and southern N.S.W. tablelands (open squares); Group B.2.2 Torrington (shaded squares); Group B3.1 (open triangles) and Group B3.2 (solid triangles). Numbered specimens are referred to in text.

Analysis 2 (specimens 1–114, characters 1–11, 14): Analysis 2 included the 114 specimens in group B of analysis 1. Characters 12 and 13 were invariate after removal of *A. buxifolia* and were deleted. Data were range standardised before reanalysis. Five groups are evident from the UPGMA classification (Fig. 4), ordination (Fig. 5) and MST: three major groups (groups B1, B2 and B3) and two pairs of subgroups (B2.1 & B2.2 and B3.1 & B3.2). The high co-phenetic correlation coefficient for the UPGMA classification indicates that the branch lengths in the dendrogram are highly correlated with the distances in the original distance matrix. Group B1 (specimens 57–66, 109–114) corresponds to *A. buckinghamii*. Groups B2 and B3 represent two different forms within *A. asteriscophora sensu lato*. Group B2 includes specimens from the Emerald district of Victoria (B2.1) and a widespread group that contains the lectotype of *A. asteriscophora* (B2.2). Group B3 includes specimens from the Armidale region of New South Wales (B3.1) and the Mt Kaputar and Mt Canobolas districts of New South Wales (B3.2). In the ordination, specimens from Torrington are positioned on the periphery of group B2.2 (typical *A. asteriscophora*), and adjacent to group B3 (Mt Kaputar and the Armidale region) indicating they have similarities to both groups. However, the Torrington specimens cluster with Victorian and southern New South Wales specimens in group B2 in the UPGMA classification (Fig. 4), which is supported by the Minimum Spanning Tree (Fig. 6).

The positions of specimens 100, 101 and 107 are problematic. Specimen 101 from Deepwater in New South Wales is positioned between B3.2 and B2.2 in the MST (Fig. 6)

but is included in B2.2 because all other specimens from this region clustered clearly within B2.2. Specimen 107 from Mt Canobolas in New South Wales is considered misplaced in the MST where it is linked to specimens of group B2.2, as it clusters within group B3.2 in the classification and ordination. Otherwise, the MST supports the groups identified in both the classification and the ordination. Specimen 100 from Cabramurra in New South Wales is clustered in group B3.2 in all analyses. However, it does not fit well in this group because, other than the high trichome density and short petiole, it is very similar to specimens in group B2.2, and all other specimens from this region are clustered within B2.2.

Means and Cramer values for the most discriminating continuous characters between groups are given in Table 2.

Table 2. Cramer values, means and ranges for the four most discriminating continuous characters between groups.

Character	Cramer Value		Group Number				
			B1.1	B2.1	B2.2	B3.1	B3.2
Petiole length (mm) (character 4)	0.7263	Mean	3.0	2.5	3.7	0.0	0.5
		Maximum	6.1	3.7	7.0	0.0	1.7
		Minimum	1.8	1.7	1.3	0.0	0.0
Peduncle length (mm) (character 7)	0.8192	Mean	1.0	4.6	7.1	5.8	5.9
		Maximum	1.7	4.9	14.3	6.3	7.7
		Minimum	0.0	3.8	4.6	5.5	4.8
Pedicel length (mm) (character 8)	0.7404	Mean	1.4	8.5	7.8	8.4	11.0
		Maximum	1.9	11.5	3.8	9.3	14.3
		Minimum	0.9	5.7	15.4	7.3	8.9
Flower number per inflorescence (Character 6)	0.8469	Mean	1.1	4.2	4.6	4.6	4.1
		Maximum	2.2	4.8	6.6	5.7	6.0
		Minimum	1.0	3.3	3.0	4.0	3.0

Group B1 (specimens 57–66, 109–114) includes two syntypes (specimens 109 and 110) of *A. buckinghamii* and is distinct from other groups in the analysis by the combination of the following features: flowers solitary or almost so (character 6); a reduced or absent peduncle (character 7; mean 1 mm); a reduced or absent pedicel (character 8; mean 1.4 mm); and petiolate leaves (character 4; mean petiole length 3 mm).

Group B2 (specimens 6–56, 72–101) may be distinguished from group B1 in combining the following features: inflorescence an umbel of three or more (character 6), a peduncle length of 3.5–15 mm (character 7), and a pedicel length of 3.5–15.5 mm (character 8); from group B3 by the longer petiole length (character 4; range 1.7–7 mm), a lower average trichome density on the adaxial leaf surface (character 5), and leaf shape.

Group B2.1 (specimens 7–11, 85–87) consists of those specimens that have white or, very rarely, pale lemon petals (character 14), short petioles (character 4; mean length 2.5 mm), and an obovate to spathulate lamina with a rounded apex.

Group B2.2 (specimens 6, 12–56, 72–84, 88–99, 101) includes the lectotype (specimen 83), and two isolectotypes (specimens 81 and 82) of *A. asteriscophora*. This group is defined by specimens with bright yellow flowers in umbels of three or more (character 6; mean 4.6), long peduncles (character 7; mean 7.1 mm), long pedicels (character 8; mean 7.8 mm) and petiolate leaves (character 4; mean petiole length 3.7 mm) that are elliptic to spathulate, with a rounded apex.

Group B3 (specimens 1–5, 67–71, 100, 102–108) is readily distinguished from group B1 in combining the following features: inflorescence an umbel of three or more (character 6), a peduncle length of 4.5–8 mm (character 7), and a pedicel length of 7–15 mm

(character 8); from group B2 by the short or absent petiole (character 4) and a higher average trichome density on the adaxial leaf surface (character 5).

Group B3.1 (specimens 67–71, 102) contains specimens distinguished by the combination of the following characters: leaves sessile or almost so (character 4), obcordate to obdeltate, margins strongly recurved (character 11), high trichome density (character 5; mean 21 trichomes per mm^2), and a shorter leaf length/distance to widest point (character 3) than all other groups.

Group B3.2 (specimens 1–5, 100, 103–108) is defined by the same characters as group B3.1, but differs in the subsessile leaves (character 4, mean petiole length 0.5 mm), and the non-recurved leaf margins (character 11).

These five groups (B1, B2.1, B2.2, B3.1 and B3.2) correspond to geographic regions. Specimens in group B1 are all from the Penrose, Wingello and Berrima districts of the central tablelands of New South Wales. Specimens in group B2.1 are all from a small area in the Emerald-Avonsleigh district of Victoria. Group B2.2 has the largest distribution, ranging from the Macedon district north of Melbourne to the southern tablelands of New South Wales. There is a disjunct occurrence of group B2.2 at Torrington in northern New South Wales. Interestingly, this population is more similar to specimens from the southern New South Wales tablelands and Victoria than it is to other northern New South Wales populations (group B3). Specimens in group B3.2 are restricted to Mt Kaputar NP in northern New South Wales, while specimens in group B3.1 occur near Palour Mountain north west of Armidale in New South Wales. The recognised groups do not overlap geographically.

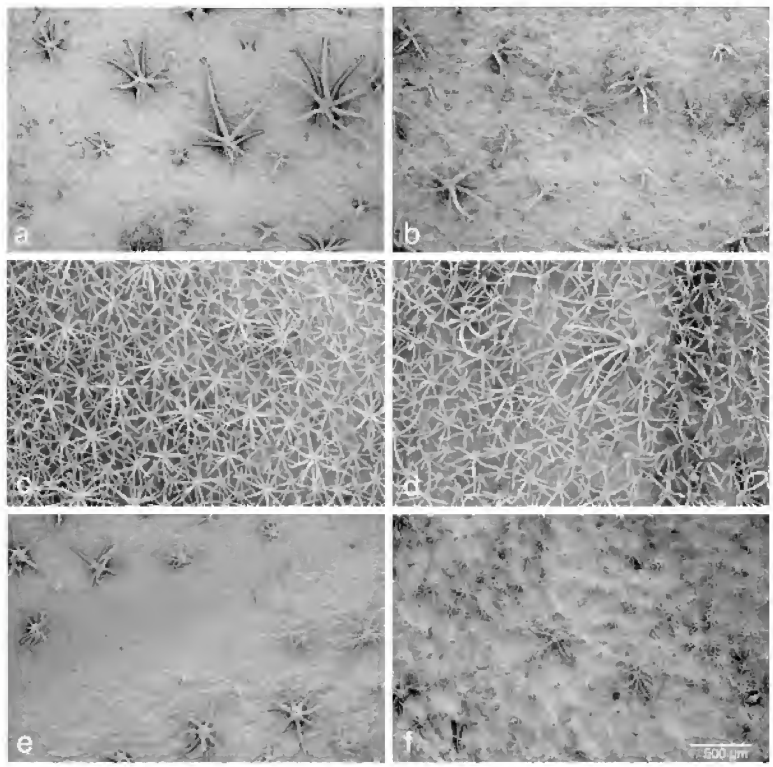

Figure 7. Adaxial leaf surfaces, illustrating variation in density of stalked, multiangular, stellate trichomes. (a – d) *Asterolasia asteriscophora sensu lato* (e – f) *A. buckinghamii*: (a) specimen 11 from Emerald; (b) specimen 77 from Wallaby Creek; (c) specimen 2 from Mt Kaputar; (d) specimen 73 from Torrington; (e) specimen 62 from Mittagong; and (f) specimen 57 from Medway.

The ordination identifies variation in trichome density, with a trend in Groups B2 and B3 of increasing density on the adaxial leaf surface correlated with decreasing latitude, although latitude per se is unlikely to be the causal factor. Specimens from the greater Melbourne region have the lowest average trichome density, while specimens from Torrington and Mt Kaputar have the highest (Fig. 7). Indumentum density correlating with latitude change was also observed in *Boronia grandisepala* F.Muell *sensu lato*. (Duretto & Ladiges 1997), where increased indumentum density correlated with increased aridity. Cultivated *A. asteriscophora* plants originating from Torrington and Emerald growing under controlled glasshouse conditions were observed to have similar trichome densities to specimens sampled from those populations in the field.

ISOZYME ANALYSIS

Four enzyme systems corresponding to seven loci were resolved. These were: AAT (one locus); FUM (two loci); GPI (two loci); and PGM (two loci). An additional locus for AAT was scored for phenotype only. The low number of enzyme systems resolved is insufficient to describe genetic diversity between populations. However, with the exception of *A. buckinghamii*, the results broadly correlate with morphological results.

Problems were encountered with the resolution of some enzyme banding patterns making interpretation of allele mobility difficult. A characteristic of the Rutaceae is the presence of aromatic volatile oils in the pellucid oil glands of the leaves. It appears that these oils and/or other unknown compounds have co-migrated with some alleles during electrophoresis making interpretation of band patterns difficult. Similar problems to those encountered in this study have been documented in other studies of Rutaceae (eg. Durham 1998). There were difficulties in particular with interpreting the AAT system. AAT is a dimeric system where a homozygote is represented by one band and a heterozygote by three. The number of bands for individuals ranged from two to four. However, it was decided to interpret the different banding patterns as phenotypes, and record the frequencies of each. This method has previously been used by Faville *et al.* (1995) in a study on allozyme variation in the grass *Agrostis capillaris*L. In addition, the need to freeze some samples reduced enzyme activity in those samples and resulted in fewer enzyme systems being satisfactorily resolved. Consequently, only results for populations from the Melbourne region are presented here, although allele frequency data for all populations are recorded in Table 3.

A Distance Wagner Tree (Fig. 8) for populations of *A. asteriscophora* in the greater Melbourne region based on four enzyme systems and seven loci (Table 3) indicates that these populations are genetically very similar. The distances in the Wagner Tree correlate

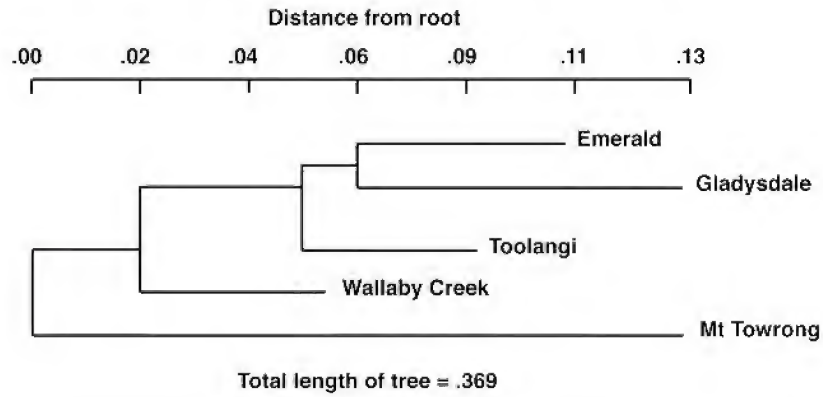

Figure 8. Wagner Tree for isozyme data rooted at the midpoint for five populations of *Asterolasia asteriscophora* in the Melbourne region. Co-phenetic correlation coefficient 0.974.

with the geographic isolation of these populations from one another. The Mt Towrong population near Macedon is the most distinct and also the most geographically isolated population. The population with white petals from Emerald is not distinct based on these four enzyme systems, but is distinct based on analysis of phenotype for AAT.

Table 3. Allele frequencies for 13 populations of *Asterolasia.* Sample size per locus = 15 individuals. Populations are: 1–7 *A. asteriscophora sensu lato* Victorian populations, 1 Emerald; 2 Gladysdale; 3 Toolangi; 4 Wallaby Creek; 5 Mt Towrong; 6 Mt Bowen; 7 Ben Cruachan Creek. 8–12 *A. asteriscophora sensu lato* New South Wales populations, 8 Mt Kaputar; 9 Torrington; 10 Mittagong; 11 Medway; 12 Tumut River. 13 *A. correifolia* Boonoo Boonoo New South Wales.

Locus	Allele	Population number												
		1	2	3	4	5	6	7	8	9	10	11	12	13
Pgm-1	1	0	0	0	0	0.466	0	0.833	0	0.5	0.04	0	0.133	0
	2	1	1	1	0.844	0.533	0.833	0.177	0.1	0.5	0.966	1	0.877	1
	3	0	0	0	0.166	0	0.177	0	0.9	0	0	0	0	0
Pgm-2	1	0	0	0	0	0.1	0	1	1	0	0	0	0	0.5
	2	1	1	1	0.893	0.9	0.714	0	0	1	1	0.8	0.846	0.5
	3	0	0	0	0.107	0	0.286	0	0	0	0	0.2	0.154	0
Gpi-1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Gpi-2	1	0	0	0	0	0	0	0	0.466	0	0	0	0	0
	2	0	0	0.033	0.2	0.2	0.233	0	0	0	0	0.7	0.1	0.5
	3	0.633	0.8	0.466	0.5	0.733	0.777	0.633	0.544	0	0.633	0	0.633	0.5
	4	1	0.166	0.266	0.1	0.033	0	0	0	0.433	0	0.044	0.1	0
	5	0.233	0	0.166	0.166	0.033	0	0.377	0	0.577	0.377	0.266	0.177	0
	6	0	0.033	0.066	0.033	0	0	0	0	0	0	0	0	0
Fum-1	1	0	0.033	0	0	0	-	-	-	-	-	-	-	-
	2	0.133	0.033	0	0	0	-	-	-	-	-	-	-	-
	3	0.867	0.933	1	1	1	-	-	-	-	-	-	-	-
Fum-2	1	0	0	0.066	0.033	0.167	-	-	-	-	-	-	-	-
	2	0.9	1	0.934	0.967	0.833	-	-	-	-	-	-	-	-
	3	0.1	0	0	0	0	-	-	-	-	-	-	-	-
Aat-1	1	1	1	1	1	1	-	-	-	-	-	-	-	-

Taxonomic conclusions

Morphological analyses have contributed to elucidating two major (groups B2 & B3) and four minor groups (groups B2.1, B2.2, B3.1 & B3.2) within *Asterolasia asteriscophora sensu stricto*, and confirm that specific status for *A. buxifolia* (group A) and *A. buckinghamii* (group B1) is appropriate. Although there is insufficient information to describe genetic diversity between populations based on isozyme analyses due to the low number of enzyme systems resolved, fixed allele differences support the morphological findings.

In the classification proposed here, Group B2 and B3 are recognised at the species level. Group B2 corresponds to *A. asteriscophora*, and the subgroups B2.1 and B2.2 are recognised here as subspecies. Group B2.2 contains the type for *A. asteriscophora*, therefore it is designated *A. asteriscophora* subsp. *asteriscophora*. Group B2.1 is recognised here as *A. asteriscophora* subsp. *albiflora* B.J.Mole. It is given sub-specific rank because, other than petal colour, it is morphologically similar to typical *A. asteriscophora*. Petal colour has been used as a character to delimit species of *Asterolasia* in Western Australia

(Wilson 1980). Specimens 85 and 87 (Fig. 4) sampled near the white-petalled population over 10 years ago, have flowers which are very pale lemon in colour, indicating specimens from this locality may grade into typical *A. asteriscophora.* During subsequent field work at these localities, however, only white-petalled individuals were observed. Some white-petalled specimens collected at this site during field work for this research (*Mole 73–77*) had discoloured to a pale lemon colour 12 months after pressing. In some cases petals may turn pale lemon on drying, however information recorded on specimen 87 would indicate flower colour for that specimen was "pale lemon" at the time of collection. Even so, it is considered by the authors and the collector of that specimen (Walsh 2001 pers. comm.) to be consistent in other characters with white flowering specimens from the same locality. *Asterolasia asteriscophora* subsp. *albiflora* is geographically isolated from the typical subspecies and commences flowering several weeks earlier than populations of the latter in the Melbourne area. This characteristic has been observed over several years (Gross 1998 pers. comm.). Isozyme phenotype differences for Aspartate aminotransferase locus 2 (*Aat-2*) distinguish *A. asteriscophora* subsp. *albiflora* from the typical subspecies.

Specimens from the Torrington region are assigned to *A. asteriscophora* subsp. *asteriscophora* as the ordination for morphological data (Fig. 5) places them with, but peripheral to, specimens in this taxon, despite their geographic proximity to group B3 described below. Both the MST and the classification also support the inclusion of Torrington specimens in subsp. *asteriscophora.* Isozyme frequency data (Table 3) also indicate that specimens from Torrington are more similar to southern populations of *A. asteriscophora* (although still distinct from them) than they are to the Mt Kaputar population (group B3.2).

Group B3 constitutes a new species, *Asterolasia rupestris* B.J.Mole, and the two subgroups within group B3 constitute subspecies. *Asterolasia rupestris* includes specimens from Mt Kaputar NP (group B3.2, specimens 1–5, 103–105), Mt Canobolas (group B3.2, specimens 106–108) and the Parlour Mountains (group B3.1, specimens 68–71, 102). *Asterolasia rupestris* differs from typical *A. asteriscophora* in having obcordate leaves and a reduced or absent petiole. *Asterolasia rupestris* subsp. *rupestris* (group B3.2), represented by specimens from Mt Kaputar and Mt Canobolas, has plane leaf margins and *A. rupestris* subsp. *recurva* B.J.Mole. (group B3.1), represented by specimens from The Parlour Mountains near Armidale, has recurved leaf margins. The differences in leaf margins were consistently distinct between specimens assigned to each subspecies. The specimens from Mt Canobolas in New South Wales are somewhat intermediate between *A. rupestris* and *A. asteriscophora* in that the leaves are narrowly obcordate. They are included in *A. rupestris* because of the short or absent petiole (character 4), high trichome density (character 5) and a leaf length:distance to widest point ratio (character 3) which is greater than that of *A. asteriscophora.*

Key to the *A. asteriscophora sensu lato* group.

1. Flowers usually solitary and subsessile (rarely in umbels of two or three and then only occasionally on each individual plant); peduncles absent or to 2 mm long, pedicel length at anthesis 0–2 mm long
   2. Adaxial surface of the leaves glabrous; abaxial surface of petals with an indumentum of sessile, globular stellate trichomes; ovary glabrous .....**1. *A. buxifolia***
   2: Adaxial surface of the leaves with an indumentum of stalked multiangular stellate trichomes; abaxial surface of petals with an indumentum of stalked multiangular stellate trichomes; ovary with an indumentum of multiangular, stellate trichomes .....**2. *A. buckinghamii***

1: Flowers in umbels of three or more (usually five); peduncles 3–15 mm long; pedicel length at anthesis 3–15 mm long
   3. Leaves sublanceolate, elliptic-obovate, or sometimes spathulate, adaxial surface sparsely or densely covered with stellate trichomes, petiole (2–)4–7mm

long; petiole terete, not appressed to the stem; the base of lamina often adaxially V-shaped in section, giving the appearance of an extended petiole..... .....**3. *A. asteriscophora***

4. Petals bright yellow.....**3a. *A. asteriscophora* subsp. *asteriscophora***

4: Petals white, rarely pale lemon .....**3b. *A. asteriscophora* subsp. *albiflora***

3: Leaves obcordate to obdeltate, adaxial surface densely covered with stellate trichomes, sessile or petiole < 2 mm long, when present somewhat thickened and flat, often appressed to the stem; the lamina base not adaxially V-shaped in section.....**4. *A. rupestris***

5. Leaf margins not recurved.....**4a. *A. rupestris* subsp. *rupestris***

5: Leaf margins strongly recurved .....**4b. *A. rupestris* subsp. *recurva***

Taxonomy

1. *Asterolasia buxifolia* Benth., *Fl. Austral.* 1: 351 (1863); *Eriostemon cunninghammii* F.Muell., *Fragm.* 9: 107 (1875). *Type*: Bells Road, Blue Mountains, N.S.W., 1834, *R.Cunningham* (lectotype K, *fide* Wilson, *Nuytsia* 12: 83–88, 1998); Botanic Gardens Sydney, 1839, *A.Cunningham s.n.* (residual syntype MEL *4546*).

Shrub to 2 m high. *Stems* with a dense indumentum of stellate trichomes. *Leaves* obovate, 10–18 mm long, 3–10 mm wide, coriaceous, apex rounded or slightly emarginate; adaxial surface glabrous; abaxial surface with an indumentum of stalked, multiangular, stellate trichomes; petiole 2–7 mm long. *Flowers* axillary, solitary; peduncles absent; pedicels 1–1.5 mm long, subtended by two white petaloid bracts. Sepals inconspicuous, c. 1 mm long. Petals five, elliptic, 6–7 mm long, yellow; abaxial surface with an indumentum of sessile, globular, stellate trichomes; adaxial surface glabrous. Stamens 10, filaments glabrous; anthers 1–1.5 mm long, each with a terminal gland. Carpels five, glabrous; style glabrous; stigma hemispherical. *Cocci* glabrous, beaked. *Seed* not seen.

Additional specimens examined: NEW SOUTH WALES: 1838, Anonymous (MEL *708653*); "Port Jackson", 1838, Theod. Scenes [sic.] ex herb. Sond. (NSW *468151*); Blue Mountains, *Vicary s.n.* (MEL *708652*, MEL *708654*); New Holland, *Anderson 52* (MEL *4827*); Blue Mountains, Hartley area, October 2000, *R.O.Makinson 1791* (CANB *n.v.*, MEL, NSW *n.v.*).

Distribution and conservation status: *Asterolasia buxifolia* was presumed extinct because it had not been located in the field since the 1830s and recent attempts to relocate it in the Blue Mountains had not been successful (Wilson 1998; Keith Ingram 1999 pers. comm.). However, a collection of the species that is consistent with the type specimen was made in October 2000 in the Hartley area of the Blue Mountains (B. Makinson pers comm.; Auld 2001; Benson & McDougall 2001). A conservation code of 2E is considered appropriate because the taxon is currently known from only one population of between 50–100 individuals (B. Makinson pers comm.).

Habitat: The species is apparently restricted to rocky watercourses, with a granitic substrate.

Phenology: Flowering specimens have been collected in October.

Etymology: The specific epithet *buxifolia* presumably means foliage like the genus *Buxus*, however there is no particular resemblance between the leaves of *A. buxifolia* and those in the genus *Buxus*.

2. *Asterolasia buckinghamii* (Blakely) Blakely, *Austral. Naturalist* 11: 12 (1941); *Phebalium buckinghamii* Blakely, *Austral. Naturalist* 10: 246 (1940). *Type citation:* "Gold Gully, Penrose, W.F.Blakely, Jeane and W.J.Buckingham, and E.Murphy, 15/10/1938*; 2 miles N.E. of Wingello railway station, same collectors, 30/9/1939.*" *Type*: Gold Gully Penrose [N.S.W], 15.x.1938, *W.F.Blakely, J. and W.J.Buckingham and*

E.Murphy (lectotype, here designated, NSW *2214*; isolectotypes MEL *232746*, CANB *81750 n.v.*); 2 miles [c. 3.2 km] NE of Wingello railway station, 30.x.1939, *W.F.Blakely, W.J.Buckingham and E.Murphy* (residual syntype NSW *468135*).

Slender upright *shrub* to 1.5 m high. *Stems* covered with an indumentum of stellate trichomes. *Leaves* orbicular to broadly obovate, 4–15 mm long, 3–7 mm wide, lateral halves adaxially concave, apex rounded to slightly emarginate; adaxial surface with a sparse to dense indumentum of stalked, multiangular, stellate trichomes (range 1–23 trichomes per mm^2); abaxial surface with a cobwebbed indumentum of stalked, multiangular, stellate trichomes; petiole 2–4 mm long. *Flowers* axillary or terminal, usually solitary, rarely in a two or three flowered umbel; peduncle 0–2 mm long; pedicel 0–2 mm long. Sepals inconspicuous, to 0.5 mm long. Petals five, elliptic, 4–8 mm long, yellow; abaxial surface with an indumentum of multiangular stellate trichomes; adaxial surface glabrous. Stamens 10, filaments glabrous; anthers 1–1.5 mm long, each with a terminal gland. Carpels 5; ovary stellate-tomentose; style glabrous; stigma hemispherical. *Cocci* beaked. *Seed* not seen.

Additional specimens examined: NEW SOUTH WALES: Medway Colliery, 10.x.1991, *M. Kennedy 153* (NSW *249646*); c. 1.5 km north of Mittagong P.O., 27.vii.1995, *S.Donaldson 560, G.Corsini and J.Toby* (CANB *9513536*); Flying Fox Road SW of Medway, 22.xii. 1995, *G.Errington 30* (NSW *264105*); Medway area, 18.x.1998, *B.J.Mole 155–160 and C.A.Mole* (*BJM155* - MEL, MELU, NSW; *BJM156–160* - MEL); Nattai River, Mittagong area, 19.x.1998, *B.J.Mole 161–165 and C.A.Mole* (*BJM161* - BRI, CANB, MEL, MELU, NSW; *BJM162–165* - MEL).

Distribution and conservation status: Asterolasia buckinghamii has been recorded from five localities (from Mittagong south to Penrose and Wingello) in the Central Tablelands of New South Wales. Two populations were located during this study at Mittagong and Medway. The population at Medway consisted of less than 30 individuals, while the population at Mittagong consisted of more than 200 individuals but was restricted to a small area, approx $100m^2$. Populations from the type locality near Penrose and Wingello were not located during this study despite searches in these areas during October and November of 1998. This region has been extensively cleared for agriculture and silviculture, and further fieldwork is recommended to establish the status of the species at this locality. A tentative conservation code of 2E is considered appropriate because the species has a geographic range of less than 100 km^2, is currently known from only two localities, and because no plants are known to occur within a conservation reserve.

Habitat: Populations from the type locality and the Nattai River north of Mittagong are recorded as growing in gullies, on river flats in sandy alluvial soils derived from sandstone, the Mittagong population also extending up a gentle north west facing slope for c. 100 m. The vegetation community at the Mittagong locality is riparian on the river flats grading to open-forest further upslope. The population at Medway, however, occurs on a south facing slope at the top of a cliff in shallow soils over sandstone in open-forest. The species therefore is not restricted to damp localities, as previously thought (Blakely 1940).

Phenology: Flowering plants have been collected from early October to late November.

Notes: This species differs from *A. asteriscophora* by the usually solitary flowers and the smaller (often absent) peduncles and pedicels, and from *A. buxifolia* by the stellate trichomes on the ovary and abaxial surface of leaves, and the type of indumentum on the abaxial petal surface.

Etymology: The specific epithet honours one of the collectors of the type specimen, Mr William J. Buckingham of Lindfield, New South Wales.

3. *Asterolasia asteriscophora* (F.Muell.) Druce, *Bot. Soc. Exch. Club Brit. Isles* 4: 606 (1917); *Phebalium asteriscophorum* F.Muell., *Trans. & Proc. Victorian Instit. Advancem.*

Sci. 31 (1855); *Asterolasia muelleri* Benth., *Fl. Austral.* 1: 350 (1863), *nom. illeg*; *Asterolasia correifolia* var. *muelleri* Maiden & Betche, *Proc. Linn. Soc. New South Wales* 26: 80 (1901). *Type:* Mt Disappointment, October 1852, *F.Mueller s.n.* (lectotype MEL *708656*, *fide* Wilson, *Nuytsia* 12: 84, 1998; isolectotypes MEL *708636*, MEL *708637*, MEL *708638*).

Slender, upright *shrub* to 2 m high. Younger *stems* densely stellate-tomentose, indumentum becoming sparse with age. *Leaves* obovate-elliptic or spathulate, 4–35 mm long, 3–15 mm wide, papery, apex rounded, rarely obtuse or slightly emarginate, flat or slightly concave adaxially; adaxial surface with a sparse or dense indumentum of multiangular, stellate trichomes (range = 1–28 trichomes per mm^2); abaxial surface cobwebbed with stalked, multiangular, stellate trichomes; petiole 2–7 mm long. Inflorescence a terminal or axillary umbel of 3–8 *flowers*; peduncles 3–11 mm long; pedicels 3–15 mm at anthesis, longer in fruit. Sepals inconspicuous, 0.5–1 mm long. Petals 5, elliptic 4–9 mm long, bright yellow, pale lemon or white; abaxial surface with an indumentum of stellate trichomes; adaxial surface glabrous. Stamens 10; filaments glabrous; anthers 1–1.5 mm long each with a terminal gland. Carpels 5; ovary stellate; style glabrous; stigma hemispherical. *Cocci* beaked. *Seed* oblong, 2–2.5 mm long, 1–1.2 mm wide, testa smooth, black and shiny; endocarp thin and brittle, a dull mustard colour, deciduous from seed (Fig 9a–h).

Distribution: This species is widely distributed, although often in small disjunct populations, along the Great Dividing Range from the Macedon and Emerald districts in Victoria, to the Tumut district in the Southern Tablelands of New South Wales. The species also occurs disjunctly in the Torrington district in the Northern Tablelands of New South Wales.

Habitat: The species is found in a range of vegetation types, including low open-forest, open-forest and riparian communities and is known to occur on a various substrates including krasnozem soils, alluvial soils, granitic sands and skeletal rocky ridgetops.

Notes: *Asterolasia asteriscophora* differs from *A. buckinghamii* by its three or more flowered umbels, and longer peduncles and pedicels, and from *A. buxifolia* by the stellate trichomes on its ovaries, stellate adaxial leaf surface, and the multiangular stellate trichomes on the abaxial surfac eof the petals.

Etymology: The specific epithet is derived from the Greek *asterios* (starry) and *phorum* (carrier) referring to the stellate trichomes found on the stems, leaves, petals and ovaries of this species.

3a. *Asterolasia asteriscophora* (F.Muell.) Druce **subsp. *asteriscophora***

Slender, upright *shrub* to 2 m high. *Leaves* obovate-elliptic or spathulate, 6–35 mm long, 3–15 mm wide, papery, apex rounded, rarely obtuse or slightly emarginate, flat or slightly conduplicate; adaxial surface with a sparse or dense indumentum of multiangular, stellate trichomes (range 1–22 trichomes per mm^2); petiole 2–7 mm long. Inflorescence an umbel of 3–8 *flowers*; peduncles 5–11 mm long; pedicels 3–15 mm at anthesis, longer in fruit. Petals elliptic 4–9 mm long, bright yellow (Fig. 9a–f).

Representative specimens examined: NEW SOUTH WALES: Tangster via Deepwater, x.1913, *J.D.Lynch* (NSW *374566*); Geehi region, 4.x.1957, *M.E.Phillips s.n. and J.Raeder-Roitysch* (NSW *262309*); Near Cabramurra, 7.xi.1961, *M.E.Phillips 99* (CANB *005413*); Gilmore Creek, Bago State Forest, 4.xi.1962, *K.Giles s.n.* (NSW *468149*); Dingo Creek, Silent Grove Road north of Torrington, ix.1971, *H.J.Wissmann s.n.* (NE *029226*); Geehi Gorge road, 1.xii.1971, *D.J.Wimbush s.n.* (NSW *261158*); Kosciusko NP Peak River, 100 m upstream of junction with Wildhorse Creek, 31.x.1993, *N.Taws 235 and A.Scott* (CANB *9314794*); Kosciusko NP, Peak River, 6.xi.1993, *N.Taws 239 and A.Scott* (CANB *9314798*, MEL *278248*); Mt Kosciusko NP, Geehi area, 14.x.1998, *B.J.Mole 133–142 and C.A.Mole* (*BJM133* - BRI, MEL, MELU, NE, NSW; *BJM134–142* - MEL); 5.7 km along Elliot Way from O'Hares Rest area towards Cabramurra, 16.x.1998, *B.J.Mole149–153 and C.A.Mole* (*BJM149* - BRI, MEL, MELU, NE, NSW; *BJM150–153* - MEL);

Blather Creek, NNE of Torrington, 21.x.1998, *B.J.Mole 177–181 and C.A.Mole* (*BJM177* - BRI, MEL, MELU, NE, NSW; *BJM178–181* - MEL). VICTORIA: Plenty Ranges, x.1902, *G.Weindorfer s.n.* (MEL *5199*); Upper Genoa River, 28.ix.1947, *N.A.Wakefield s.n.* (MEL *543047*); Upper Genoa River, far East Gippsland, 29.ix.1947, *N.A.Wakefield s.n.* (MEL *709144*); Upper Genoa River, 25.ix.1948, *N.A.Wakefield 3201* (MEL *543046*); Wallaby Creek, x.1952, *D.H.Ashton s.n.* (MELU *14864*); Cascades, Kinglake West, 11.x.1962, *A.M.Gill s.n.* (MELU *19798*); Pine Mt, 17.xi.1964, *J.H.Willis s.n.* (MEL *709146*); Far north-east, Black Mt top of Mount Burrowa, 17.xi.1971, *J.H.Willis s.n.* (MEL *502061*); Mt Bowen, East Gippsland, 28.xi.1984, *D.Parkes EG217* (MEL *678435*); Burrowa-Pine Mt NP, 14.xi.1988, *F.E.Davies 677, M.J.Winsbury and S.Donaldson* (CANB *8804043*, MEL *228142*); East Gippsland, Yambulla Creek, 8.ix.1988, *D.E.Albrecht 3699* (MEL *268505*); Yambulla Creeek, 1 km upstream of its confluence with Genoa River, 9.ix.1988, *N.G.Walsh 2126, D.E.Albrecht and J.Westaway* (MEL *268564*); Pine Mtn, 20.ix.1998, *B.J.Mole 71* (MEL); Hazeldene Road south of Gladysdale, 8.x.1998, *B.J.Mole 78–82* (*BJM78* - CANB, MEL, MELU, NE, NSW; *BJM79–82* - MEL); Toolangi, Chum Creek Road, 8.x.1998, *B.J.Mole 85–89* (*BJM85* - CANB, MEL, MELU, NE, NSW; *BJM86–89* - MEL); west side of Mt Towrong, Mt Macedon Regional Park, 11.x.1998, *B.J.Mole 90–94 and C.A.Mole* (*BJM90* - CANB, MEL, MELU, NSW; *BJM91–94* - MEL); Ben Cruachan Creek NW of Ben Cruachan summit, 12.x.1998 *B.J.Mole 98, 100–103 and J.B.Mole* (*BJM98* - CANB, MEL, MELU, NE, NSW; *BJM100–103* - MEL); East Gippsland, Pheasant Creek Track, 12.x.1998, *B.J.Mole 111–119 and J.B.Mole* (*BJM111* - CANB, MEL, MELU, NSW; *BJM112–119* - MEL); East Gippsland, Warbisco Track [Mt Bowen], 13.x.1998, *B.J.Mole 123–132 and J.B.Mole* (*BJM123* - CANB, MEL, MELU, NSW; *BJM124–132* - MEL); Mt Kosciusko NP, Geehi area, 14.x.1998, *B.J.Mole 133–142 and J.B.Mole* (*BJM133* - CANB, MEL, MELU, NSW; *BJM134–139* - MEL); Mt Buffalo NP, Eurobin Creek Falls, 16.x.1998, *B.J.Mole 144 and C.A.Mole* (MEL, NSW).

Distribution and conservation status: *Asterolasia asteriscophora* subsp. *asteriscophora* is widely distributed, although usually in small disjunct populations, particularly along the Great Dividing Range from the Macedon and Gladysdale districts in Victoria, to the Tumut district in the New South Wales southern tablelands. A disjunct occurrence of the subspecies is known from the Torrington district in northern New South Wales. Populations from Woods Point and the upper Genoa River (Victoria), as indicated by herbarium records, could not be located despite searching in those areas during October and November of 1998. The subspecies is widespread with several populations in national parks and others in proclaimed conservation reserves, and appears to be secure.

Phenology: Flowering specimens have been collected from October to November. Populations of this subspecies in the Melbourne region were observed to commence flowering 3–4 weeks later than *A. asteriscophora* subsp. *albiflora*

3b. ***Asterolasia asteriscophora*** subsp. ***albiflora*** B.J.Mole, *subsp. nov.*

A subspecie typica foliis et floribus generaliter parvioribus, foliis obovatis-spathulatis, pedunculis saepe brevioribus et petalis albis differt.

It differs from the typical subspecies in the generally smaller leaves and flowers, obovate - spathulate leaves, frequently smaller peduncles, and white petals.

Type: Victoria, Emerald - Avonsleigh Road, Southern end of Country Club golf course, 8.x.1998, *B.J.Mole 73* (holotype MEL *2120867*; isotypes CANB, MEL *2068578*, MELU, NE, NSW).

Eriostemon spathulifolius Gand., *Bull. Soc. Bot. France* 60: 458 (1913). Type citation: "Australia, Victoria ad Esmerald [probably Emerald], *MacLennan, n.v.* (possibly at LYON *fide* Paul G. Wilson pers. comm.). Equated with *A. asteriscophora* 'form of *Asterolasia* found at Emerald...' by Wilson (1970, p. 120).

Slender, upright *shrub* to 1.5 m high. *Leaves* obovate to spathulate, 4–16 mm long, 3–12 mm wide, flat, apex rounded, adaxial surface with a sparse (1–3 trichomes per mm^2) indumentum of multiangular, stellate trichomes; lamina papery; petiole 1–4 mm long. Inflorescence an umbel of 3–5 *flowers*; peduncles 3–5 mm long; pedicels 6–15 mm at

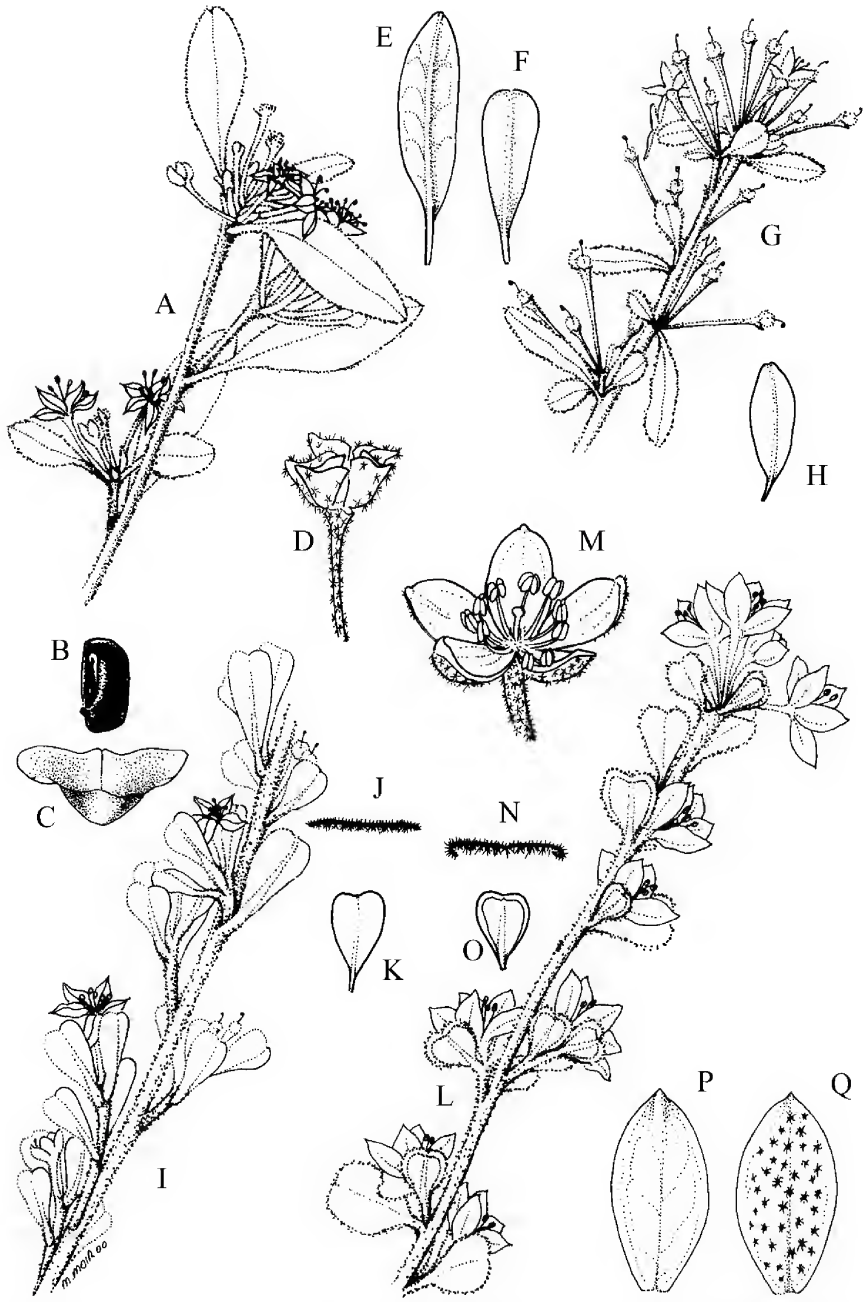

Figure 9. **a–e** *Asterolasia asteriscophora* subsp. *asteriscophora* (*Mole 100*): **a** Flowering sprig × 1; **b** Seed × 5; **c** Endocarp × 5; **d** Fruits × 4; **e** Leaf (abaxial view) × 1. **f** Leaf of *A. asteriscophora* subsp *asteriscophora* (*Mole 177*). **g–h** *A. asteriscophora* subsp. *albiflora* (*Mole 75*): **g** Flowering sprig × 1; **h** Leaf (abaxial view) × 1.5. **i–k** *A. rupestris* subsp. *rupestris* (*Fox s.n.* CANB *406009*): **i** Flowering sprig × 1; **j** Leaf section × 2; **k** Leaf (abaxial view) × 1.5. **l–q** A. *rupestris* subsp. *recurva* (*Mole 172*): **l** Flowering sprig × 1; **m** Flower × 2; **n** Leaf section × 2; **o** Leaf (abaxial view) × 1.5; **p** Petal (adaxial view) × 2.5; **q** Petal (abaxial view) × 2.5.

anthesis, longer in fruit. Petals elliptic, 4–6 mm long, white, rarely pale lemon (Fig. 9g–h).

Representative specimens examined: VICTORIA: Emerald, 27.x.1906, *P.R.H.St.John s.n.* (NSW *262302*); Dandenong Ranges, Belgrave, January 1933, *J.H.Willis s.n.* (MEL *709148*); between Beaconsfield and Emerald, 28.ix.1933, *T.S.Hart s.n.* (MEL *709145*); Dandenong Ranges, between Emerald and Avonsleigh, 7.x.1934, *J.H.Willis s.n.* (MEL *709149*); Dandenong Ranges, Emerald-Clematis, 5.x.1945, *A.C.Beauglehole 7016* (MEL *2101021*); Upper Ferntree Gully [locality uncertain, Hemphill pers. comm. 1998], 23.ix.1965, *B.Hemphill s.n.* (MEL *2101022*); Eastern Highlands, Emerald-Monbulk Road, 24.ix.1993, *N.G.Walsh 3507* (MEL *2020508*); Emerald-Avonsleigh Road, southern end of Country Club golf course, 8.x.1998, *B.J.Mole 74–77* (MEL).

Synonymy: *Eriostemon spathulifolius* is probably a synonym of *Asterolasia asteriscophora* subsp. *albiflora* but as the type material was not seen this can not be verified (cf. Wilson 1970; McGillivray 1973). The type for *Eriostemon spathulifolius* is probably at LYON (P.G. Wilson pers. comm. 1998) and was requested in 1998 but has not been located.

Distribution and conservation status: This subspecies is known only from three localities in the Emerald - Avonsleigh district of Victoria, all of which are threatened by urban development. Two of these are located in residential areas, while the third is located within a quarry site. It was previously recorded from Menzies Creek, 'Paradise' and Clematis. The single record from the Grampians (ix.1937, *C. French Jnr. s.n.,* MEL *709143)* is most likely incorrectly labelled. No other record of *A. asteriscophora* is known from the Grampians. A conservation code of 2Ei is appropriate; the subspecies is known from a geographic range of less than 100 km, is in serious risk of disappearing from the wild within 10–20 years if present residential development and associated pressures continue, and is not known to occur within a conservation reserve.

Phenology: This subspecies flowers from early October to late November and has been observed to commence flowering 3–4 weeks earlier than the typical subspecies.

Etymology: The subspecific epithet is derived from the Latin, *albus* (white) and *floreo* (to flower), alluding to the white colour of the petals.

4. *Asterolasia rupestris* B.J.Mole, *sp. nov.*

*Asterolasiae asteriscophora*e (F.Muell.) Druce affinis sed foliis generaliter brevioribus et subsessilibus vel saepe sessilibus, lamina obcordata vel obdeltata et supra dense stellato-tomentosam differt.

Similar to *Asterolasia asteriscophora* but differs in the generally shorter leaves, which are subsessile, or frequently sessile, the obcordate - obdeltate lamina, and the adaxial lamina surface, which is densely stellate.

Type: New South Wales, walking track to the Governor, Mt Kaputar NP, 27.xi.1987, *J.M. Fox s.n.* (holotype CANB *406009*).

Upright *shrub* to 1.5 m tall. *Stems* with a stellate indumentum. *Leaves* shortly petiolate, or frequently sessile; lamina obcordate or obdeltate, 9–20 mm long, 6–15 mm wide, papery, apex emarginate, sometimes truncate, base attenuate, slightly conduplicate, margins recurved or flat; adaxial surface with a dense indumentum of hyaline multiangular stellate trichomes (15–31 trichomes per mm^2); abaxial surface cobwebbed with stalked, multiangular stellate trichomes; petiole when present somewhat thickened and flat, often appressed to the stem. Inflorescence a terminal or axillary umbel of 3–5 *flowers*; peduncles 4–9 mm long; pedicels 6–15 mm long. Sepals inconspicuous, 0.5–1 mm long. Petals 5, elliptic, 5–9 mm long, yellow, abaxial surface with an indumentum of rust coloured stellate trichomes; adaxial surface glabrous. Stamens 10; filaments glabrous; anthers 1–2 mm long, each with a terminal gland. Carpels 5, stellate-tomentose; style glabrous; stigma hemispherical. *Cocci* beaked. *Seed* not seen (Fig. 9i–q).

Distribution: This species is only known from Mt Kaputar and Mt Lindsay in Mt Kaputar NP east of Narrabri, and from near Parlour Mountain northwest of Armidale; all

of these populations are apparently restricted to trachyte outcrops. Collections have been made from Mt Canobolas, southwest of Orange, but is possibly extinct at this location (see notes under *A. rupestris* subsp. *rupestris*).

Phenology: The flowering period for the species is from late September to early November.

Notes: The species was previously included in *A. asteriscophora* (e.g. Porteners 1991; Wilson 1998) from which it can be readily distinguished by the obcordate to obdeltate leaf shape and sessile to subsessile leaves. Specimens from the Mt Kaputar area lodged at CANB have been determined as *Asterolasia sp. nov.* by I.R. Telford and A. Lynne.

Etymology: The specific epithet, which is derived from the Latin *rupestris*, means rock dwelling, and refers to the rocky outcrops to which this species appears to be confined.

4a. ***Asterolasia rupestris*** B.J.Mole subsp. ***rupestris***

Leaves plane. Inflorescence an umbel of 3–5 *flowers*; peduncles 4–6 mm long; pedicels 7–10 mm long. Petals 5–8 mm long (Fig.9i–k).

Representative specimens examined: NEW SOUTH WALES: Mt Lindsay, Nandewar Mountains, 5.xi.1909, *R.H.Cambage s.n.* (NSW *262327*); Springside via Orange, 10.x.1948, *W.E.Giles s.n.* (NSW *262271*); Springside, near Orange, 14.x.1956, *W.E.Giles s.n.* (NSW *468148*); Devils Hole [Mt Canobolas], Towac, 7 miles SW of Orange, 8.xi.1960, *E.F.Constable s.n.* (MEL *2100530*, NE *29227*, NSW *52954*); Eckfords (sic) Lookout, Mt Kaputar NP, 4.ix.1976, *G.L.Harden s.n.* (NE *38917*); The Governor, Mt Kaputar NP, 21.xi.1976, *R.Coveny 8937 and S.K. Roy* (NSW *468152*, CANB *373743*); Eckards Lookout, Mt Kaputar NP, 25.xi.1987, *J.M. Fox 87/111* (CANB *406306*); Mt Kaputar NP, "The Governor", 15.ix.1998, *B.J. Mole 41–51 and W.A. Gebert* (*BJM41* - CANB, MEL, MELU, NE, NSW; *BJM42–51* - MEL).

Distribution and conservation status: This subspecies has been collected from only a small number of localities at Mt Lindsay and Mt Kaputar in Mt Kaputar National Park. A population recorded from Mt Canobolas during the 1950s was not located during this study despite searching in that area during October 1998. The locality recorded on herbarium specimens is now farmland and/or pine plantations, and so, at this locality the taxon is possibly extinct. Further searches of remnant vegetation in the Mt Canobolas area are required to establish the status of the taxon at this locality. A conservation code of 3E is considered appropriate.

Habitat: The subspecies grows in heathlands, shrublands and low open forest on skeletal gravelly soils on trachyte outcrops, tending to favour sheltered positions but also found in exposed sites.

4b. ***Asterolasia rupestris*** subsp. ***recurva*** B.J.Mole, *subsp. nov.*

A subspecie typica lamina recurva differt.

It differs from the typical subspecies in the recurved lamina margins.

Type: New South Wales, northwest of Armidale, 4.2 km north east of Parlour Mtn, 20.x.1998, *B.J.Mole 171 and C.A.Mole* (holotype MEL *2120868*; isotypes CANB, MEL *2068579*, MELU, NE, NSW).

Leaf margins recurved. Inflorescence an umbel of 3–6 *flowers*; peduncles 4–8 mm long; pedicels 8–15 mm long. Petals 6–7 mm long (Fig. 9l–q).

Additional specimens examined: NEW SOUTH WALES: The Parlour, about 6 miles from Booralong, 28.x.1951, *G.L.Davies s.n.* (NSW *374569*); 4.2 km NE of Parlour Mt, 20.x.1998, *B.J.Mole 172–175 and C.A.Mole* (MEL).

Distribution and conservation status: This subspecies is known only to the authors from one locality near Parlour Mountain north west of Armidale, New South Wales. The population is on private land, which the landholders wish to manage for conservation purposes (Brian Hardaker & Jeremy Bruhl, 1998 pers. comm.). Approximately 30–40 plants

were observed at the locality, restricted to an area c. 50 m^2 along a small creek. Further searches in the Parlour Mountain district are required to establish the extent of this taxon. A conservation code of 2E is appropriate as the taxon has a geographic range of less than 100 km^2 and is known only from two collections, both outside a conservation reserve.

Habitat: The subspecies grows in low open forest on skeletal gravelly soils, along gullies.

Etymology: The subspecific epithet refers to the recurved margins of the leaves, characteristic of this subspecies.

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Appendix 1. Specimens and data used in the phenetic analyses of *Asterolasia asteriscophora sensu lato*. Principal collector given only. For continuous characters (see table 1), mean values are shown. Missing data is coded as 999.

Specimen Number	*Collector & Number*	Herbarium & Sheet	Locality Number	CharacterNumber 1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*B.J.Mole 41*	MEL	Mt Kaputar NSW	12.30	1.51	1.33	0.80	19.00	3.50	999	999	6.17	2.19	1	0	1	1
2	*B.J.Mole 44*	MEL	Mt Kaputar NSW	12.10	1.59	1.18	0.50	23.20	3.20	999	999	6.30	1.73	1	0	1	1
3	*B.J.Mole 45*	MEL	Mt Kaputar NSW	13.90	2.07	1.28	0.90	19.60	3.00	999	999	6.60	2.17	1	0	1	1
4	*B.J.Mole 49*	MEL	Mt Kaputar NSW	13.80	1.54	1.23	0.90	19.80	3.75	999	999	4.75	2.38	1	0	1	1
5	*B.J.Mole 51*	MEL	Mt Kaputar NSW	13.00	1.48	1.19	0.70	29.80	3.00	999	999	999	999	1	0	1	1
6	*B.J.Mole 71*	MEL	Pine Mtn. Vic	25.40	2.67	1.52	3.30	10.80	999	999	999	999	999	1	999	999	999
7	*B.J.Mole 73*	MEL	Emerald Vic	16.10	2.50	1.40	2.20	1.60	4.60	3.80	11.50	5.10	1.91	1	0	1	0
8	*B.J.Mole 74*	MEL	Emerald Vic	13.60	2.40	1.78	3.00	2.80	4.80	4.90	8.00	5.50	2.25	1	0	1	0
9	*B.J.Mole 75*	MEL	Emerald Vic	14.40	2.23	1.48	2.30	0.80	4.50	4.80	10.20	4.70	2.16	1	0	1	0
10	*B.J.Mole 76*	MEL	Emerald Vic	12.20	1.66	1.60	3.70	1.80	4.60	4.60	8.00	4.70	1.99	1	0	1	0
11	*B.J.Mole 77*	MEL	Emerald Vic	10.00	1.88	1.18	2.80	1.40	4.00	4.70	6.80	4.50	2.25	1	0	1	0
12	*B.J.Mole 78*	MEL	Gladysdale Vic	16.90	2.72	2.20	3.50	4.80	5.33	5.40	9.10	4.10	1.79	1	0	1	1
13	*B.J.Mole 79*	MEL	Gladysdale Vic	13.10	2.83	1.83	3.90	2.60	4.33	8.20	8.40	5.40	2.38	1	0	1	1
14	*B.J.Mole 80*	MEL	Gladysdale Vic	11.80	2.22	1.93	4.00	4.40	3.20	6.40	6.40	4.30	2.28	1	0	1	1
15	*B.J.Mole 81*	MEL	Gladysdale Vic	19.70	2.81	2.40	5.00	2.60	3.75	6.00	11.00	4.30	1.98	1	0	1	1
16	*B.J.Mole 82*	MEL	Gladysdale Vic	22.20	2.42	1.71	5.00	2.40	4.50	5.10	9.40	4.70	1.89	1	0	1	1
17	*B.J.Mole 85*	MEL	Toolangi Vic	27.10	3.82	2.09	5.30	1.40	5.00	5.40	8.00	5.00	2.23	1	0	1	1
18	*B.J.Mole 86*	MEL	Toolangi Vic	27.80	2.84	2.13	5.20	2.20	4.67	7.00	7.30	5.00	2.30	1	0	1	1
19	*B.J.Mole 87*	MEL	Toolangi Vic	28.30	3.73	2.00	4.80	2.20	4.80	6.70	13.00	6.90	2.53	1	0	1	1
20	*B.J.Mole 88*	MEL	Toolangi Vic	27.40	2.83	2.06	4.10	0.60	4.75	6.10	11.80	6.80	2.63	1	0	1	1
21	*B.J.Mole 89*	MEL	Toolangi Vic	20.80	2.84	2.17	4.80	1.00	5.17	7.60	5.90	5.40	1.98	1	0	1	1
22	*B.J.Mole 90*	MEL	Mt Macedon Vic	21.10	2.17	1.50	4.80	2.80	4.50	999	999	8.13	2.33	1	0	1	1
23	*B.J.Mole 91*	MEL	Mt Macedon Vic	24.70	2.45	1.35	3.50	5.60	5.17	999	999	6.80	1.82	1	0	1	1
24	*B.J.Mole 92*	MEL	Mt Macedon Vic	19.20	1.36	1.46	2.80	1.40	4.00	5.00	5.25	6.70	2.17	1	0	1	1
25	*B.J.Mole 93*	MEL	Mt Macedon Vic	23.60	1.84	1.34	4.30	4.60	3.75	7.67	4.67	7.70	1.81	1	0	1	1
26	*B.J.Mole 94*	MEL	Mt Macedon Vic	23.90	2.02	1.62	5.00	2.60	5.33	999	7.80	7.25	1.89	1	0	1	1
27	*B.J.Mole 98*	MEL	Ben Cruachan Vic	29.10	2.61	2.90	6.60	9.80	5.50	9.20	7.30	6.10	2.06	1	0	1	1

Specimen Number	*Collector & Number*	Herbarium & Sheet	Locality Number	CharacterNumber 1	2	3	4	5	6	7	8	9	10	11	12	13	14
28	*B.J.Mole 100*	MEL	Ben Cruachan Vic	33.90	2.90	2.39	6.40	6.20	4.50	9.70	10.40	5.40	2.38	1	0	1	1
29	*B.J.Mole 101*	MEL	Ben Cruachan Vic	29.40	3.05	2.67	6.80	7.40	5.86	7.70	9.20	4.90	2.25	1	0	1	1
30	*B.J.Mole 102*	MEL	Ben Cruachan Vic	26.30	2.71	2.43	6.20	5.60	5.00	8.60	8.50	4.90	2.35	1	0	1	1
31	*B.J.Mole 103*	MEL	Ben Cruachan Vic	30.40	2.74	2.30	7.00	4.20	4.60	7.40	10.20	4.50	1.88	1	0	1	1
32	*B.J.Mole 111*	MEL	Pheasant Creek Vic	19.70	2.63	1.74	2.50	12.20	3.67	6.30	7.90	7.00	2.47	1	0	1	1
33	*B.J.Mole 112*	MEL	Pheasant Creek Vic	17.00	2.61	1.63	3.40	19.20	3.67	6.00	7.00	6.30	2.27	1	0	1	1
34	*B.J.Mole 114*	MEL	Pheasant Creek Vic	18.50	2.54	1.57	3.30	12.40	5.00	6.40	7.40	5.20	1.80	1	0	1	1
35	*B.J.Mole 115*	MEL	Pheasant Creek Vic	15.40	2.15	1.61	1.40	15.60	3.83	7.10	7.70	5.10	2.00	1	0	1	1
36	*B.J.Mole 119*	MEL	Pheasant Creek Vic	18.20	2.53	1.74	2.90	22.00	4.00	6.50	7.80	7.00	2.21	1	0	1	1
37	*B.J.Mole 123*	MEL	Mt Bowen Vic	16.30	2.01	1.47	2.80	11.60	3.20	5.50	4.10	5.30	2.07	1	0	1	1
38	*B.J.Mole 124*	MEL	Mt Bowen Vic	20.80	2.61	1.71	2.70	14.20	3.20	6.80	4.10	6.40	2.44	1	0	1	1
39	*B.J.Mole 125*	MEL	Mt Bowen Vic	19.50	2.83	1.37	1.30	13.80	3.29	7.60	7.70	4.90	1.71	1	0	1	1
40	*B.J.Mole 126*	MEL	Mt Bowen Vic	16.30	1.70	1.67	4.00	10.80	3.80	6.80	4.10	5.70	1.74	1	0	1	1
41	*B.J.Mole 129*	MEL	Mt Bowen Vic	14.20	1.72	1.63	3.00	19.40	3.60	6.80	6.00	5.50	1.79	1	0	1	1
42	*B.J.Mole 133*	MEL	Geehi NSW	27.50	3.57	1.69	2.50	11.00	4.60	4.67	4.60	5.60	2.01	1	0	1	1
43	*B.J.Mole 135*	MEL	Geehi NSW	26.70	3.12	1.80	4.50	10.40	5.40	7.33	4.80	4.60	1.86	1	0	1	1
44	*B.J.Mole 136*	MEL	Geehi NSW	32.40	3.70	1.83	6.40	10.00	5.60	6.40	7.50	5.80	1.90	1	0	1	1
45	*B.J.Mole 137*	MEL	Geehi NSW	30.10	3.05	1.98	3.10	10.80	6.63	999	5.00	4.50	2.05	1	0	1	1
46	*B.J.Mole 139*	MEL	Geehi NSW	33.50	4.06	1.90	3.00	10.60	5.00	6.50	4.50	5.00	1.78	1	0	1	1
47	*B.J.Mole 144*	MEL	Mt Buffalo Vic	30.70	3.18	2.00	4.20	13.00	4.00	10.00	6.00	6.50	1.75	1	0	1	1
48	*B.J.Mole 145*	MEL	Mt Buffalo Vic	30.10	3.36	2.54	3.60	10.80	4.75	9.00	11.60	4.50	2.03	1	0	1	1
49	*B.J.Mole 146*	MEL	Mt Buffalo Vic	37.80	4.52	1.76	4.40	13.40	3.67	6.67	7.30	4.90	2.17	1	0	1	1
50	*B.J.Mole 147*	MEL	Mt Buffalo Vic	37.20	3.37	1.83	3.50	10.20	4.00	7.00	6.90	5.10	1.93	1	0	1	1
51	*B.J.Mole 148*	MEL	Mt Buffalo Vic	33.40	3.94	1.48	4.00	13.60	4.50	999	12.00	6.30	1.65	1	0	1	1
52	*B.J.Mole 149*	MEL	Tumut River NSW	27.00	3.22	1.52	3.00	7.40	4.88	7.20	9.80	8.20	2.45	1	0	1	1
53	*B.J.Mole 150*	MEL	Tumut River NSW	24.60	2.77	1.83	3.50	5.00	5.89	11.30	9.60	8.00	1.90	1	0	1	1
54	*B.J.Mole 151*	MEL	Tumut River NSW	29.70	4.50	1.63	3.00	6.80	5.17	9.00	8.20	9.00	2.29	1	0	1	1
55	*B.J.Mole 152*	MEL	Tumut River NSW	27.60	3.29	1.56	2.60	5.20	4.00	8.00	9.00	8.20	1.98	1	0	1	1
56	*B.J.Mole 153*	MEL	Tumut River NSW	29.70	3.24	1.75	4.00	6.20	5.17	8.00	11.40	7.20	2.00	1	0	1	1
57	*B.J.Mole 155*	MEL	Medway NSW	14.20	1.90	1.44	4.00	23.00	1.15	1.70	1.30	6.30	2.06	1	0	1	1

Specimen Number	*Collector & Number*	Herbarium & Sheet	Locality Number	CharacterNumber 1	2	3	4	5	6	7	8	9	10	11	12	13	14
58	*B.J.Mole 157*	MEL	Medway NSW	11.90	1.73	1.59	2.90	22.00	1.00	1.40	1.90	7.20	1.97	1	0	1	1
59	*B.J.Mole 158*	MEL	Medway NSW	15.20	1.96	1.80	6.10	18.80	1.00	1.60	1.90	6.70	2.05	1	0	1	1
60	*B.J.Mole 159*	MEL	Medway NSW	10.80	1.74	1.32	3.60	16.20	1.00	0.60	1.90	5.63	2.53	1	0	1	1
61	*B.J.Mole 160*	MEL	Medway NSW	9.80	1.42	1.56	2.90	14.60	1.00	1.50	1.60	6.20	1.90	1	0	1	1
62	*B.J.Mole 161*	MEL	Mittagong NSW	11.20	2.13	1.70	3.20	1.60	1.09	0.80	0.90	5.30	1.91	1	0	1	1
63	*B.J.Mole 162*	MEL	Mittagong NSW	8.60	2.38	1.52	3.20	2.20	2.20	1.10	1.60	5.00	2.27	1	0	1	1
64	*B.J.Mole 163*	MEL	Mittagong NSW	8.60	1.77	1.37	1.90	1.00	1.14	1.10	1.00	6.30	2.36	1	0	1	1
65	*B.J.Mole 164*	MEL	Mittagong NSW	10.90	1.95	1.50	2.70	3.20	1.00	0.90	1.00	5.80	2.34	1	0	1	1
66	*B.J.Mole 165*	MEL	Mittagong NSW	10.60	1.69	1.54	3.20	1.00	1.07	1.30	1.70	7.10	2.06	1	0	1	1
67	*B.J.Mole 171*	MEL	Parlour Mtns NSW	13.90	2.24	1.11	0.00	15.80	4.25	5.50	9.30	7.40	2.01	0	0	1	1
68	*B.J.Mole 172*	MEL	Parlour Mtns NSW	11.70	1.70	1.21	0.00	22.80	4.00	6.30	9.10	7.50	1.88	0	0	1	1
69	*B.J.Mole 173*	MEL	Parlour Mtns NSW	12.40	1.88	1.21	0.00	12.00	4.60	6.00	7.30	6.00	1.73	0	0	1	1
70	*B.J.Mole 174*	MEL	Parlour Mtns NSW	13.90	1.66	1.06	0.00	24.40	4.80	5.80	8.50	8.00	2.00	0	0	1	1
71	*B.J.Mole 175*	MEL	Parlour Mtns NSW	12.80	1.57	1.09	0.00	22.40	5.75	6.00	7.80	7.00	2.07	0	0	1	1
72	*B.J.Mole 177*	MEL	Torrington NSW	24.30	3.67	1.40	4.40	21.80	5.00	8.80	6.90	6.70	2.59	1	0	1	1
73	*B.J.Mole 178*	MEL	Torrington NSW	23.90	3.05	1.38	3.40	21.20	5.80	9.90	10.40	6.50	1.90	1	0	1	1
74	*B.J.Mole 179*	MEL	Torrington NSW	25.70	3.38	1.18	3.90	20.40	5.50	10.60	7.50	6.30	2.35	1	0	1	1
75	*B.J.Mole 180*	MEL	Torrington NSW	19.60	2.67	1.19	2.20	16.60	4.86	11.50	7.60	6.20	2.32	1	0	1	1
76	*B.J.Mole 181*	MEL	Torrington NSW	24.60	2.58	1.29	2.90	16.60	5.00	14.30	8.00	6.30	1.87	1	0	1	1
77	*D.H.Ashton s.n.*	MELU *14864*	Wallaby creek Vic	21.00	2.73	1.82	3.70	3.00	4.00	6.50	5.90	5.20	2.01	1	0	1	1
78	*M.Gill s.n.*	MELU *19798*	Wallaby creek Vic	10.50	2.13	1.51	2.50	3.40	4.60	7.00	5.20	4.80	2.03	1	0	1	1
79	*M.Gill s.n.*	MELU *19802*	Wallaby creek Vic	13.20	2.75	1.75	3.20	4.20	5.00	6.60	4.60	5.40	2.20	1	0	1	1
80	*Anon*	MELU *19803*	Wallaby creek Vic	23.40	3.16	1.82	4.10	5.60	4.00	5.50	8.70	6.00	2.24	1	0	1	1
81	*F.Mueller s.n.*	MEL *708637*	Mt Dissapointment Vic	14.20	2.66	1.56	2.80	2.40	3.80	5.20	4.40	4.50	1.98	1	0	1	1
82	*F.Mueller s.n.*	MEL *708638*	Mt Dissapointment Vic	9.90	2.11	1.76	2.60	4.80	3.25	5.00	4.50	4.40	2.33	1	0	1	1
83	*F.Mueller s.n.*	MEL *708656*	Mt Dissapointment Vic	6.30	2.85	1.71	2.20	2.20	3.00	5.90	3.80	3.25	2.17	1	0	1	1
84	*G.Weindorfer 185*	MEL *5199*	Plenty Ranges Vic	22.00	3.06	2.19	3.20	1.80	4.80	5.80	10.50	5.10	2.55	1	0	1	1
85	*B.Hemphill s.n.*	MEL s.n.	Upper Ferntree Gully Vic	9.50	2.45	1.61	1.70	2.00	3.33	4.40	9.00	4.10	2.32	1	0	1	0.5
86	*J.H.Willis s.n.*	MEL *709148*	Belgrave Vic	8.40	1.76	1.54	1.90	1.20	3.60	4.90	8.70	4.30	2.43	1	0	1	0
87	*N.G.Walsh 3507*	MEL *2020508*	Emerald Vic	10.80	2.42	1.60	2.20	2.80	4.33	4.50	5.70	3.80	2.00	1	0	1	0.5

Specimen Number	*Collector & Number*	Herbarium & Sheet	Locality Number	CharacterNumber 1	2	3	4	5	6	7	8	9	10	11	12	13	14
88	*J.H.Willis s.n.*	MEL *502061*	Black Mtn Vic	16.60	2.45	1.66	1.80	13.00	5.67	8.30	10.60	5.50	2.05	1	0	1	1
89	*F.E.Davies 677*	CBG *8804043*	Pine Mtn Vic	21.10	3.44	1.49	1.60	10.20	3.00	6.33	5.40	7.00	2.32	1	0	1	1
90	*J.H.Willis s.n.*	MEL *709146*	Pine Mtn Vic	22.50	3.35	1.73	2.50	10.60	5.00	6.40	11.40	6.30	2.41	1	0	1	1
91	*N.G.Walsh 2126*	MEL *268504*	Upper Genoa River Vic	22.80	3.50	2.14	4.60	12.80	5.00	5.40	7.00	4.67	1.62	1	0	1	1
92	*D.E.Albrecht 3699*	MEL *268505*	Upper Genoa River Vic	25.70	2.68	1.57	6.30	6.40	5.00	5.90	999	6.60	2.34	1	0	1	1
93	*N.A.Wakefield 3261*	MEL *543047*	Upper Genoa River Vic	20.80	3.18	1.67	3.50	15.20	5.00	6.30	4.50	5.00	2.50	1	0	1	1
94	*N.A.Wakefield s.n.*	MEL *709 144*	Upper Genoa River Vic	24.00	3.80	1.66	3.30	11.40	5.67	7.20	6.60	3.80	2.00	1	0	1	1
95	*N.A.Wakefield 3201*	MEL *543046*	Upper Genoa River Vic	16.00	1.90	1.41	3.60	12.80	5.20	5.50	9.80	5.10	2.55	1	0	1	1
96	*M.E.Phillips s.n.*	NSW *262309*	Geehi NSW	19.80	2.31	2.13	4.10	13.20	4.25	999	999	999	999	1	999	999	999
97	*D.J. Wimbush s.n*	CANB *261158*	Geehi NSW	25.88	2.98	1.79	2.75	10.20	4.33	5.30	15.40	5.60	1.70	1	0	1	1
98	*N.Taws 235*	CBG *9314794*	Peak River NSW	27.90	4.12	1.73	1.80	1.40	5.75	5.20	13.00	8.10	2.47	1	0	1	1
99	*N.Taws 239*	CBG *9314798*	Peak River NSW	37.80	5.12	2.05	1.90	1.00	4.50	5.80	10.60	6.00	2.32	1	0	1	1
100	*M.E.Phillips 99*	CBG *005413*	Cabramurra NSW	18.70	2.78	1.85	1.70	28.60	3.00	5.10	14.30	5.60	2.36	1	0	1	1
101	*J.D.Lynch s.n.*	NSW *374566*	Deepwater NSW	18.00	2.65	1.19	3.40	23.60	4.86	4.60	6.60	5.00	2.17	1	0	1	1
102	*G.L.Davis s.n.*	NSW *374569*	Parlour Mtns NSW	12.90	2.19	1.11	0.00	31.00	4.00	5.50	999	999	999	0	0	1	1
103	*R.Coveny 8937*	NSW *468152*	Mt Kaputar NSW	17.30	2.67	1.19	0.00	22.00	5.67	5.00	9.20	6.00	2.02	1	0	1	1
104	*M.Kennedy 477*	NSW *262289*	Mt Kaputar NSW	15.50	1.74	1.20	0.00	26.00	4.33	4.80	999	6.13	2.35	1	0	1	1
105	*R.H.Cambage s.n.*	NSW *262327*	Mt Lindsay NSW	16.30	2.56	1.12	0.00	25.00	6.00	5.80	8.90	5.50	2.15	1	0	1	1
106	*E.F.Constable s.n.*	NSW *52954*	Mt Canobolas NSW	17.70	2.16	1.26	0.20	16.60	5.40	7.60	11.70	6.70	2.25	1	0	1	1
107	*W.E.Giles s.n.*	NSW *468148*	Mt Canobolas NSW	20.90	3.57	1.14	0.60	13.20	4.00	7.67	999	8.20	1.96	1	0	1	1
108	*W.E.Giles s.n.*	NSW *262271*	Mt Canobolas NSW	20.50	3.40	1.17	1.00	25.60	5.00	5.50	999	7.50	2.14	1	0	1	1
109	*W.F.Blakeley s.n.*	NSW *2214*	Penrose NSW	7.10	1.50	1.37	2.10	1.40	1.00	0.40	1.80	4.83	2.67	1	0	1	1
110	*W.F.Blakeley s.n.*	MEL *232746*	Penrose NSW	8.00	1.71	1.49	2.60	3.20	1.00	0.70	1.10	4.50	1.98	1	0	1	1
111	*W.J.Buckingham s.n*	NSW *468135*	Wingello NSW	8.00	1.60	1.50	2.10	1.00	1.00	1.40	1.00	5.40	2.42	1	0	1	1
112	*S.Donaldson 560*	CBG *951 3536*	Mittagong NSW	7.80	2.36	1.32	1.80	1.40	1.00	0.70	1.00	4.40	2.02	1	0	1	1
113	*M.Kennedy 153*	NSW *249646*	Medway NSW	10.70	1.68	1.77	3.50	7.80	1.00	0.00	1.80	6.30	2.17	1	0	1	1
114	*G.Errington 30*	NSW *264105*	Medway NSW	13.80	2.58	1.25	2.20	17.60	1.00	999	999	999	999	1	999	999	999
115	*A.Cunningham s.n.*	MEL *4546*	Blue Mtns NSW	13.80	1.87	1.42	3.00	0.00	1.00	0.00	1.00	6.00	3.00	1	1	0	1
116	*R.Cunningham s.n.*	KEW	Blue Mtns NSW	14.30	1.85	1.57	3.40	0.00	1.00	0.00	1.50	7.00	3.36	1	1	0	1